PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q

A2

(11) International Publication Number: **WO 99/06587**

(43) International Publication Date: 11 February 1999 (11.02.99)

(21) International Application Number: PCT/EP98/04836

(22) International Filing Date: 3 August 1998 (03.08.98)

(30) Priority Data:
97113319.4 1 August 1997 (01.08.97) EP

(71) Applicant *(for all designated States except US)*: MORPHOSYS GESELLSCHAFT FÜR PROTEINOPTIMIERUNG AG [DE/DE]; Am Klopferspitz 19, D–82152 Martinsried (DE).

(72) Inventors; and

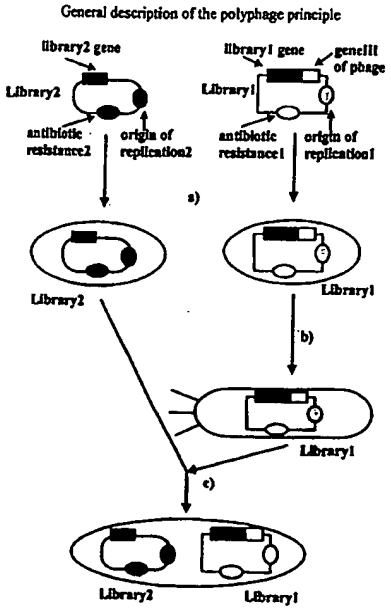
(75) Inventors/Applicants *(for US only)*: RUDERT, Fritz [DE/DE]; Josef–Retzer–Strasse 36, D–81241 München (DE). GE, Liming [CN/DE]; Portiastrasse 12, D–81545 München (DE). ILAG, Vic [PH/DE]; Knorrstrasse 85, D–89897 München (DE).

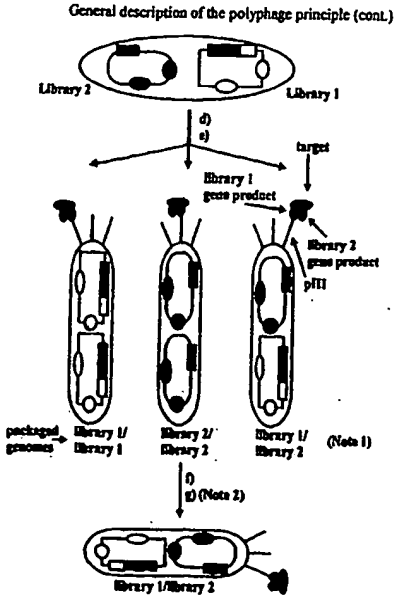
(74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, D–81675 München (DE).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS OF A MULTIMERIC (POLY)PEPTIDE COMPLEX





(57) Abstract

The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS OF A MULTIMERIC (POLY)PEPTIDE COMPLEX

The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.

Since its first conception by Ladner in 1988 (WO88/06630), the principle of displaying repertoires of proteins on the surface of phage has experienced a dramatic progress and has resulted in substantial achievements. Initially proposed as display of single-chain Fv (scFv) fragments, the method has been expanded to the display of bovine pancreatic trypsin inhibitor (BPTI) (WO90/02809), human growth hormone (WO92/09690), and of various other proteins including the display of multimeric proteins such as Fab fragments (WO91/17271; WO92/01047).

A Fab fragment consists of a light chain comprising a variable and a constant domain (VL-CL) non-covalently binding to a heavy chain comprising a variable and constant domain (VH-CH1). In Fab display one of the chains is fused to a phage coat protein, and thereby displayed on the phage surface, and the second is expressed in free form, and on contact of both chains, the Fab assembles on the phage surface.

Various formats have been developed to construct and screen Fab phage-display libraries. In its simplest form, just one repertoire, e. g. of heavy chains, is encoded on the phage or phagemid vector. A corresponding light chain, or a repertoire of light chains, is expressed separately. The Fab fragments assemble either inside a host cell, if the light chain is co-expressed from a plasmid, or outside the cell in the medium, if a collection of secreted phage particles each displaying a heavy chain is contacted with the light chain(s) expressed from a different host cell. By screening such Fab libraries, just the information about the heavy chain encoded on the phage or phagemid vector is retrievable, since that vector is packaged in the phage particle. By reverting the format and displaying a library of light chains, and

assembling Fab fragments by co-expressing or adding one or more of the heavy chains identified in the first round, corresponding light chain-heavy chain pairs can be identified.

To avoid that multi-step procedure, both repertoires may be cloned into one phage or phagemid vector, one chain expressible as a fusion with at least part of a phage coat protein, the second expressible in free form. After selection, the phage particle will contain the sequence information about both chains of the selected Fab fragments. The disadvantage of such a format is that the overall complexity of the library is limited by transformation efficiency. Therefore, the library size will usually not exceed 10^{10} members.

For various applications, a library size of up to 10^{14} would be advantageous. Therefore, methods of using site-specific recombination, either based on the Cre/lox system (WO92/20791) or on the attλ system (WO 95/21914) have been proposed. Therein, two collection of vectors are sequentially introduced into host cells. By providing the appropriate recombination sites on the individual vectors, recombination between the vectors can be achieved by action of an appropriate recombinase or integrase, achieving a combinatorial library, the overall library size being the product of the sizes of the two individual collections. The disadvantages of the Cre/lox system are that the recombination event is not very efficient, it leads to different products and is reversible. The attλ system leads to a defined product, however, it creates one very large plasmid which has a negative impact on the production of phages. Furthermore, the action of recombinase or integrase most likely leads to undesired recombination events.

Thus, the technical problem underlying the present invention is to develop a simple, reliable system which enables the simultaneous identification of members of a multimeric (poly)peptide complex, such as the identification of heavy and light chain of a Fab fragment, in phage display systems.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims. Accordingly, the present invention allows to easily create and screen large libraries of multimeric (poly)peptide complexes for properties such as binding to a target, as in the case of screening Fab fragment libraries, or such as enzymatic activity, as in the case of libraries of multimeric enzymes. The technical approach of the present invention, i.e. the retrieval of information about two members of a multimeric (poly)peptide complex

encoded on two different vectors without requiring a recombination event, is neither provided nor suggested by the prior art.

Accordingly, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, said method being characterized by screening or selecting for polyphage particles that contain said combination.

Surprisingly, it has been achieved by the present invention that the phenomenon of polyphages can be used to co-package the genetic information of two or more members of multimeric (poly)peptide complexes in a phage display system. The occurrence of polyphage particles has been observed 30 years ago (Salivar et al., Virology 32 (1967) 41-51), where it was described that approximately 5% of a phage population form particles which are longer than unit length and which contain two or more copies of phage genomic DNA. They occur naturally when a newly forming phage coat encapsulates two or more single-stranded DNA molecules. In specific cases, it has been seen that co-packaging of phage and phagemids or single-stranded plasmid vectors takes place as well (Russel and Model, J. Virol. 63 (1989) 3284-3295). Despite of occasional scientific articles about the morphogenesis of polyphage particles, a practical application has never been discussed or even been mentioned. In WO92/20791 in example 26, a model experiment for a combinatorial Fab display library expressed from separate vectors is presented. However, there is only a screening process for either of the two vectors described. Thus, the prior art teaches away from screening for the simultaneous presence of two vectors in a polyphage particle.

In the context of the present invention, the term "multimeric (poly)peptide complex" refers to a situation where two or more (poly)peptide(s) or protein(s), the "members" of said multimeric complex, can interact to form a complex. The interaction between the individual members will usually be non-covalent, but may be covalent, when post-translational modification such as the formation of disulphide-bonds between any two members occurs. Examples for "multimeric (poly)peptide complexes" comprise structures such as fragments derived from immunoglobulins (e. g. Fv, disulphide-linked Fv (dsFv), Fab fragments), fragments derived from other members of the immunoglobulin superfamily (e.g. α,β-

heterodimer of the T-cell receptor), and fragments derived from homo-or heterodimeric receptors or enzymes. In phage display, one of said members is fused to at least part of a phage coat protein, whereby that member is displayed on, and assembly of the multimeric complex takes place at, the phage surface. A "combinatorial phage library" is produced by randomizing at least two members of said multimeric (poly)peptide complex at least partially on the genetic level to create two libraries of genetically diverse nucleic acid sequences in appropriate vectors, by combining the libraries in appropriate host cells and by achieving co-expression of said at least two libraries in a way that a library of phage particles is produced wherein each particle displays one of the possible combinations out of the two libraries.
By screening such a combinatorial phage library displaying multimeric (poly)peptide complexes for a predetermined property, a collection of phage particles will be identified. Partially, these particles will just contain the genetic information of one of the members of the multimeric complex. The inventive principle of the present invention is the screening step for polyphage particles containing the genetic information of a combination of library members.

Furthermore, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, comprising the steps of

(a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to, and displayed at, the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;

(b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules of said second library are able to be packaged in a phage particle and carry

or encode a second selectable and/or screenable property different from said first property;

(c) optionally, providing nucleic acid sequences encoding further members of a multimeric (poly)peptide complex;

(d) expressing members of said libraries of recombinant vectors mentioned in steps (a), (b), and optionally nucleic acid sequences mentioned in step (c), in appropriate host cells under appropriate conditions, so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;

(e) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;

(f) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;

(g) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (f);

(h) identifying said combination of nucleic acid sequences.

Optionally, further members of said multimeric complex may be provided in the case of ternary, quaternary or higher (poly)peptide complexes. These further members may, for example, be co-expressed from one of the phage or phagemid vectors or from a separate vector such as a plasmid. Even libraries of such further members could be employed in which case further screenable or selectable properties would have to be introduced on the corresponding vectors. Alternatively, such further libraries could be contained in said first of second libraries of recombinant vector molecules. In another option, further screening and/or selection steps or a repetition of the individual steps can be carried out, to optimize the result of obtaining and identifying said nucleic acid sequences.

Furthermore, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, comprising the steps of

(a) expressing in appropriate host cells under appropriate conditions

(aa) genetically diverse nucleic acid sequences contained in a first library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to and displayed at the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;

(ab) genetically diverse nucleic acid sequences contained in a second library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;

(ac) optionally, nucleic acid sequences encoding further members of a multimeric (poly)peptide complex,

so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;

(b) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;

(c) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;

(d) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (c);

(e) identifying said combination of nucleic acid sequences.

In a preferred embodiment of the method of the present invention, the vectors of said first and said second library are a combination of a phage vector and a phagemid vector.

In a further preferred embodiment of the method of the present invention, the vectors of said first and said second library are a combination of two phagemid vectors, said appropriate conditions comprising complementation of phage genes by a helper phage.

In a most preferred embodiment of the method of the present invention said two phagemid vectors are compatible.
The term "compatibility" refers to a property of two phagemids to be able to coexist in a host cell. Incompatibility is connected to the presence of incompatible plasmid origins of replication belonging to the same incompatibility group. An example for compatible plasmid origins of replication is the high-copy number origin ColE1 and the low-copy number origin p15A.

Therefore, in a further preferred embodiment of the method of the present invention, said two phagemid vectors comprise a ColE1 and a p15A plasmid origin of replication.

In a most preferred embodiment of the method of the present invention, said two phagemid vectors comprise a ColE1 and a mutated ColE1 origin.
It could be shown, that two phagemids both having a ColE1-derived plasmid origin of replication can coexist in a cell as long as one of the ColE1 origins carries a mutation.

Particularly preferred is a method, wherein said vectors and/or said helper phage comprise different phage origins of replication.

Most preferred is an embodiment of the method of the present invention, wherein said phage vector, said phagemid vector(s) and/or said helper phage are interference resistant.
The term "interference" refers to a property that phagemids inhibit the production of progeny phage particles by interfering with the replication of the DNA of the phage. "Interference resistance" is a property which overcomes this problem. It has been found that mutations in the intergenic region and/or in gene II contribute to interference resistance (Enea and Zinder, Virology 122 (1982), 222-226; Russel et al., Gene 45 (1986) 333-338). It was identified that phages called IR1 and IR2 (Enea and Zinder, Virology 122 (1982), 222-226), and mutants derived therefrom such as R176 (Russel and Model, J. Bacteriol. 154 (1983) 1064-1076), R382, R407 and R408 (Russel et al., Gene 45 (1986) 333-338) and R383 (Russel and Model, J. Virol. 63 (1989) 3284-3295) are interference resistant by carrying mutations in the untranslated region upstream of gene II and in the gene II coding region.

Therefore, in a preferred embodiment of the method of the present invention, said phage vector, said phagemid vector(s) and/or said helper phage have mutations in the phage intergenic region(s), preferably in positions corresponding to position 5986 of f1, and/or in gene II, preferably in positions corresponding to position 143 of f1.

In a most preferred embodiment said phage vector, said phagemid vector(s) and/or said helper phage are, or are derived from, IR1 mutants such as R176, R382, R383, R407, R408, or from IR2 mutants.

In a further embodiment or the method of the invention, said vectors and/or said helper phage comprise hybrid nucleic acid sequences of f1, fd, and/or M13 derived sequences.

In the context of the present invention, the term "hybrid nucleic sequences" refers to vector elements which comprise sequences originating from different phage(mid) vectors.

Surprisingly, it has been found that a vector constructed combining a part derived from fd phage and a second part derived from R408, a derivative of f1 phages, is interference resistant and additionally, gives predominantly polyphage particles.
Therefore, a most preferred embodiment of the method of the present invention relates to a vector which is, or is derived from, fpep3_1B-IR3seq with the sequence listed in Figure 4.

In a yet further preferred embodiment of the method according to the present invention, said derivative is a phage comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

The invention relates in an additional preferred embodiment to a method, wherein said derivative is a phagemid comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

The invention relates in a further preferred embodiment to a method, wherein said derivative is a helper phage comprising essentially the phage origin or replication from fpep3_1B-

IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Most preferred is an embodiment of the method of the invention, wherein said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

The formation of polyphage particles has been examined in more detail by different groups. It was found that amber mutations in genes VII and IX lead to the amplified production of infectious polyphage particles (Lopez and Webster, Virology 127 (1983) 177-193). A couple of mutants in gene VII (R68, R100) and in gene IX (N18) were identified and further characterized.
Accordingly, in a preferred embodiment of the method of the present invention, the gene VII contained in any of said vectors contains an amber mutation, and most preferably, said mutation is identical to those found in phage vectors R68 or R100.
Further preferred is an embodiment, wherein the gene IX contained in any of said vectors contains an amber mutation, and most preferably said mutation is identical to that found in phage vector N18.

Several phage coat proteins have been used in displaying foreign proteins including the gene III protein (gIIIP), gVIp, and gVIIIp.
In a preferred embodiment of the method of the present invention, said phage coat protein is gIIIp or gVIIIp.

In a particularly preferred embodiment of the method of the present invention, said phage particles are infectious by having a full-length copy of gIIIp.
The gIIIp is a protein comprising three domains. The C-terminal domain is responsible for membrane insertion, the two N-terminal domains are responsible for binding to the F pilus of *E. coli* (N2) and for the infection process (N1).

In a most preferred embodiment of the method of the invention, said phage particles are non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the

displayed multimeric (poly)peptide complexes with a corresponding partner coupled to an infectivity-mediating particle.

In the context of the present invention, the term "infectivity-mediating particle" (IMP) refers to a construct comprising either the N1 domain or the N1-N2 domain. On interaction with a non-infectious phage lacking said domains, infectivity of the phage particles can be restored. The interaction between the non-infectious phage and the IMP can be mediated by a ligand fused to the IMP, which can bind to a partner displayed on the phage. By screening a non-infectious phage display library against a target ligand-IMP construct, restoration of infectivity can be used to select target-binding library members.

In a further preferred embodiment of the method of the invention, said truncated gIIIp comprises the C-terminal domain of gIIIp.

In a yet preferred embodiment of the method of the invention, said truncated gIIIp is derived from phage fCA55.

In addition to the work by Lopey and Webster cited above, Crissman and Smith (Virology 132 (1984) 445-455) could show, that the phage fCA55 which has a large deletion in gene III removing the N-terminal domains and a large part of the C-terminal domain leads exclusively to the formation of polyphages.

Particularly preferred is an embodiment of the method of the invention, wherein said predetermined property is binding to a target.

In a preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of an immunoglobulin superfamily member.

In a most preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of an immunoglobulin.

In a further most preferred embodiment of the method of the invention, said fragment is an Fv, dsFv or Fab fragment.

An additional preferred embodiment of the present invention relates to a method, wherein said predetermined property is the activity to perform or to catalyze a reaction.

In a preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is an enzyme.

In a most preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of a catalytic antibody.

In a further most preferred embodiment of the method of the invention, said fragment is an Fv, dsFv or Fab fragment.

An additional preferred embodiment of the invention relates to a method, wherein selectable and/or screenable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

In a most preferred embodiment of the method of the invention, said generation of said first and second screenable and/or selectable property is achieved after infection of appropriate host cells by said collection of phage particles.

Particularly preferred is a method, wherein said identification of said nucleic acid sequences is effected by sequencing.

Further preferred is a method, wherein said host cells are E.coli XL-1 Blue, K91 or derivatives, TG1, XL1kann or TOP10F.

An additional preferred embodiment of the invention relates to a polyphage particle which

(a) contains

(i) a first recombinant vector molecule that comprises a nucleic acid sequence, which encodes a fusion protein of a first member of a multimeric (poly)peptide complex

fused to at least part of a phage coat protein, and that carries or encodes a first selectable and/or screenable property, and

(ii) a second recombinant vector molecule that comprises a nucleic acid sequence, which encodes a second member of a multimeric (poly)peptide complex, and that carries or encodes a second selectable and/or screenable property different from said first property;

and (b) displays said multimeric (poly)peptide complex at its surface.

A most preferred embodiment of the invention relates to a polyphage particle, wherein said phage coat protein is the gIIIp.

A further preferred embodiment of the present invention relates to a polyphage particle which is infectious by having a full-length copy of gIIIp present, either in said fusion protein, or in an additional wild-type copy.

Additionally, the invention relates to a polyphage particle which is non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complex with a corresponding partner coupled to an infectivity-mediating particle.

Most preferably, the invention relates to the phage vector fpep3_1B-IR3seq with the sequence listed in Figure 4.

Additionally preferred, the invention relates to a phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Further preferred is an embodiment of the invention, which relates to a phagemid vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Preferably, the invention relates to a helper phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Additionally preferred is an embodiment, said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

Further preferred is the use of any of the vectors according to the present invention in the generation of polyphage particles containing a combination of at least two different vectors.

Most preferred is the use of vectors of the invention, wherein said combination of different vectors comprises nucleic acid sequences encoding members of a multimeric (poly)peptide complex.

Further preferred in the present invention is the use of vectors, wherein said combination of different vectors comprises nucleic acid sequences encoding interacting (poly)peptides/proteins.

Legends to Figures:

Figure 1: General description of the polyphage principle for the display of a Fab library: e.g. library 1: library of VL chains; library 2: VH chains; both libraries on compatible phagemids; in a: libraries are transformed into host cells; in b: library 1 is rescued by a helper phage; in c: libraries are combined by infection; in d: co-expression of heavy and light chains; in e: rescue by helper phages, production of phage particles, assembly of Fab on phage, selection for target; note 1: A certain fraction of the phage particles will be normal unit-lenght particles containing just one of the two genomes (not shown in Figure 1). Furthermore, polyphage does not discriminate which genomes to package. Therefore, the combinations shown in Figure 1 can arise. To select for

correctly packaged genomes, the subsequent steps are required; in f: infect host cells; in g: select for ability to confer resistance to two antibiotics to infected cells; note 2: only phage that satisfy condition according to g) represent polyphage particles which contain the correct combination of heavy and light chain of binding Fabs (Hetero-polyphage). Unit-length phage as well as polyphage carrying two identical genomes will confer only resistance to one antibiotics.

Figure 2: Functional map and sequence of phage vector fhag1A

Figure 3: Functional map and sequence of phage vector fjun_1B

Figure 4: Functional map and sequence of phage vector fpep3_1B-IR3seq

Figure 5: Compatibility of various phage and phagemid vectors: co-transformation of different vector pairs and growth in liquid culture (can/amp selection):
A. fjun_1B-R408-IR/pIG10_pep10; B. fjun_1B/pIG10_pep10 (only 1 colonie); C. fpep3_1B-IR3/pIG10_pep10; D. fjun_1B-R408-IR/pOK1Djun; E. fjun_1B/ pOK1Djun: no growth; F. fpep3_1B-IR3/pOK1Djun;
a. fjun_1B; b. fjun_1B-R408-IR; c. fpep3_1B-IR3; d. pIG10_pep10; e. pOK1Djun

Figure 6: co-transformation of positive (pep3/p75ICD combination, lane 9) and negative (jun/p75ICD, lane 10) pairs; lane 1 to 8: SIP transductants

Figure 7: Sensitivity of SIP hetero-polyphage system for selection in solution: #SIP hetero-polyphage transductants, transducing units (t.u.)/ml, produced by co-cultures of co-transformants as in Figure 6 mixed at the indicated ratios.

Figure 8: PCR to identify phage vector(s) present in SIP polyphage transductants: lane 1 to 6: SIP polyphage transductants; lane A: fpep3_1B-IR3/pIG10.3-IMPp75 co-transformant; lane B: fjun_1B-IR3/pIG10.3-IMPp75 co-transformant

Figure 9: IR Phage and Phagemid are Co-packaged into Polyphages: 1: ΔgIII phage + gIII plasmid; 2: IR phage+ phagemid

Figure 10: SIP Information is Co-transduced by Polyphages: a: IMPp75 on phage vector; b: pep10-gIII-CT fusion on phage vector; c: IMPp75 on phagemid vector; d: pep10-gIII-CT fusion on phagemid vector

The examples illustrate the invention

Example 1: Selection for polyphage transductants

In WO92/01047, page 83, a model experiment for a two-vector system is described which uses a phage vector (fd-CAT2-IV) encoding a light chain and a phagemid vector (pHEN1-III) encoding a heavy chain. The phagemid, grown in E. coli HB2151, was rescued with fd-CAT2-IV phage, and functional phage(mid)s produced. By infecting TG1 cells and plating on tetracycline (to select for fd-CAT) and ampicillin (to select for pHEN1), the ratio of phage and phagemid being packaged was determined.

By repeating this experiment, but plating on TYE plates with both antibiotics, polyphage transductants transducing both resistances simultaneously can be selected, and the genetic information contained on the phage and phagemid vector can be retrieved.

By replacing the single light and heavy chain in the constructs mentioned above by corresponding repertoires, a library of Fab-displaying phage particles can be produced. By screening that library against an immobilized target, a collection of phage particles can be identified. Polyphage particles contained in that collection can be identified by transducing both resistances as described above.

Example 2: Generation and use of an interference-resistant filamentous phage to co-package the genetic information of co-displayed interacting proteins

Introduction

The physical connection of randomly combined genetic information is of vital importance in processes such as interactive screening of two libraries of expressed protein members or for co-expression and co-display of protein pairs which are dependent on the interaction with each other for proper function.

2.1.: Construction of a interference resistant filamentous phage:

2.1.1.: Construction of fjun_1B:

- fhag1A (see Figure 2)

a. The phage vector f17/9-hag (Krebber *et al.*, 1995, *FEBS Letters* 377, 227-231) is digested with EcoRV and XmnI. The 1.1 kb fragment containing the anti-HAG Ab gene is isolated

by agarose gel electrophoresis and purified with a Qiagen gel extraction kit. This fragment is ligated into a pre-digested pIG10.3 vector (EcoRV-XmnI). Ligated DNA is transformed into DH5a cells and positive clones are verified by restriction analysis. The recombinant clone is called **pIGhag1A**. All cloning described above and subsequently are according to standard protocols (Sambrook *et al.*, 1989, *Molecular Cloning: a Laboratory Manual*, 2nd ed.)

b. The vector f17/9-hag (Krebber *et al.*, 1995) is digested with EcoRV and StuI. The 7.9 kb fragment is isolated and self-ligated to form the vector **fhag2**.

c. The chloramphenicol resistance gene (CAT) assembled *via* assembly PCR (Ge and Rudolph, *BioTechniques* 22 (1997) 28-29) using the template pACYC (Cardoso and Schwarz, *J. Appl. Bacteriol.* 72 (1992) 289-293) is amplified by the polymerase chain reaction (PCR) with the primers:

CAT_BspEI(for): 5' GAATGCTCATCCGGAGTTC

CAT_Bsu36I(rev): 5' TTTCACTGGCCTCAGGCTAGCACCAGGCGTTTAAG

d. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with BspEI and Bsu36I then ligated into pre-digested fhag2 vector (BspEI-Bsu36I; 7.2 kb fragment) to form **fhag2C**.

e. The vector fhag2C is digested with EcoRI and the ends made blunt by filling-in with Klenow fragment. The flushed vector is self-ligated to form vector **fhag2CdelEcoRI**.

f. pIGhag1A is digested with XbaI and HindIII. The 1.3 kb fragment containing the anti-HAG gene fused with the C-terminal domain of filamentous phage pIII protein is isolated and ligated with a pre-digested fhag2CdelEcoRI phage vector (XbaI-HindIII; 6.4 kb) to create the vector **fhag1A.**

- fjun_1B (see Figure 3)

a. The DNA encoding the C-terminal domain including the long linker separating it from the amino terminal domain of the filamentous phage pIII (gIII short) is amplified by PCR using pOK1 (Gramatikoff *et al.*, *Nucleic Acids Res.* 22 (1994) 5761-5762) as template with the primers:

gIII short(for): 5'GCTTCCGGAGAATTCAATGCTGGCGGCGGCTCT3'

gIII short(rev): 5'CCCCCCCAAGCTTATCAAGACTCCTTATTACG3'

b. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with EcoRI and HindIII, then ligated into pre-digested fhag1A vector (EcoRI-HindIII) to form the vector **fjun_1B.**

2.1.2.: Construction of fjun_1B-R408IR:

In order to introduce mutations which have been described to confer an interference resistance phenotype (Enea and Zinder, Virology 122 (1982), 222-226) into the non-interference resistant fd phage vector fjun_1B (see Fig.3), a 1.7 kb fragment of helper phage R408 (Stratagene) comprising the region between the unique restriction sites *Dra*III and *Bsr*GI was PCR amplified by assembly PCR. Subfragments of the 1.7 kb DraIII/BsrGI fragment were amplified from the f1 phage R408 template DNA with primer combinations FR604/FR605 and FR606/FR607 to introduce via the partially complementary primers FR605 and FR606 an additional gII mutation found to be present in the recipient construct fjun_1B. Resulting PCR fragments were gel-purified and combined to serve as template in an subsequent assembly PCR with primers FR604 and FR607. PCR conditions were standard, with approx. 25 ng template, 10 pmole of each primer, 250 pmole of each dNTP, 2 mM Mg, 2.5 U Pfu DNA polymerase (Stratagene). Amplification was done for 30 cycles, with 1 min denaturation at 94 C, 1 min annealing at 50°C, 1 min extension at 72°C. The correct-sized 1.7 kb assembly PCR product was gel-purified, digested with DraIII and BsrGI and cloned into DraIII/BsrGI-digested fjun_1B, generating fjun_1B-R408IR.

Primers: FR604 5′ GTTCACGTAGTGGGCCATCG 3′
FR605 5′ TGAGAGGTCTAAAAAGGCTATCAGG 3′
FR606 5′ TAGCCTTTTTAGACCTCTCAAAAATAG 3′
FR607 5′ CGGTGTACAGACCAGGCGC 3′

2.2.: Proof of principle experiments

Despite of the absence of the two originally associated IR mutations, the hybrid phage vector fjun_1B-R408IR (carrying the chloramphenicol acetytransferase confering chloramphenicol resistance) could be co-transformed with a phagemid (pOK1deltajun, carrying the beta-lactamase gene confering ampicilin resistance) containing a phage origin of replication. More importantly, fjun_1B-R408IR could stably co-exist with the phagemid pOK1deltajun, and the phagemid was efficiently co-packaged together with the fjun_1B-R408IR phage genome into polyphage particles. Titers of polyphages, simultaneously

transducing chloramphenicol and ampicilin resistance, reached 6 x 10^8 transducing units (t.u.)/ml of overnight bacterial culture K91 plating cells, a number almost equivalent to a titer of 10^9/ml seen after selection on chloramphenicol only. Selection of the K91 transductants on ampicilin only gave a titer of 5 x 10^9/ml. These titers indicated that more than 50 % of all phages containing fjun_1B-R408IR also contained the phagemid pOK1deltajun, thus representing polyphages. This high ratio of polyphages was confirmed by restriction analysis of transductants which had been selected on chloramphenicol only. More than 50 % of these clones also contained the phagemid in addition to the fjun_1B-R408IR phage genome. fjun_1B-R408IR was isolated in pure form from an individual transductant, which contained only this phage. The construct fjun_1B-R408IR was used with pOK1deltajun for co-transformation of DH5α cells, in order to produce selectively-infective phages (SIP) via fos-jun leucine zipper interaction (which non-covalently restores wt gIII function). Stable, double-resistant co-transformants were obtained with this combination and individual clones were grown overnight in the presence of cam/amp. The culture supernatant of these clones was filtered through a 45 μM membrane filter and used to infect exponentially-growing F+ bacteria (K91 strain) for 20 min at 37 C. To test for the presence of infective SIP polyphages the cells were plated on LB agar plates containing cam and amp and plates were incubated at 37 C overnight. Approx. 500 to 1000 transforming units (t.u.)/ml resulting in double-resistant transductants were obtained from individual co-transformants. DNA of those transductants was analyzed by restriction analysis which showed that 95 % (15/16 clones) of the clones had the correct pattern expected for fjun_1B-R408IR and pOK1deltajun. Supernatants of several polyphage transductants were tested for persistent SIP phage production by re-infection of K91 cells. This confirmed that polyphage transductants continued to produce infective SIP phages and restriction analysis of the resulting 2nd round polyphage transductants showed that 44 % (14/32 clones) contained the correct vector combination. The rest of the clones contained the correct pOK1deltajun phagemid plus a recombined phage vector with a restored wt gIII, indicating an increase in recombination frequency when both vectors are propagated in the rec+ strain K91 (compared to the rec- strain DH5α used for co-transformation of IR phage and phagemid). To test other protein-protein interactions which give a higher titer of infective SIP phages and to verify the presence of hetero-polyphages (co-packaging of phage and phagemid instead of co-infection by monophages or homo-polyphages) , two peptide ligands (previously selected by SIP, WO97/32017)

which bind to the p75 rat neurotrophin receptor (Chao et al., Science 232 (1986) 518-521) intracellular domain (p75ICD) were cloned as N-terminal gIIIc fusions in fjun_1B-R408IR (replacing jun) and the phagemid pIG10.3, leading to constructs fpep3_1B-IR3seq and pIG10.3-pep10 (WO97/32017), respectively, which contain the peptide pep3: 5'-TGTATTGTTTATCATGCTCATTATCTTGTTGCTAAGTGT-3' encoding the amino acid sequence (CysIleValTyrHisAlaHisTyrLeuValAlaLysCys) instead of the jun sequence. Sequencing of the respective parts of the transferred R408 fragment in fpep3_1B-IR3seq revealed that neither of the two IR mutations (the G5986>A mutation from complementation group I in the gII 5´non-translated region, which should be found at position 3225 in fpep3_1B-IR3seq, and the C143>T mutation (3789 in fpep3_1B-IR3seq) from complementation group II leading to a Thr>Ile amino acid exchange in gII) were found to be present. However; the gII mutation G6090>T (3329 in fpep3_1B-IR3seq), leading to a Leu>Val exchange, introduced by assembly PCR was present. Furthermore, three additional mutations compared to an f1 phage could be identified: G5737>A (2976 in fpep3_1B-IR3seq) in the phage origin of replication, G343>A (3989) in gII, and G601>T (4247) in gII/X.

The functional map and the sequence of fpep3_1B-IR3seq are given in Figure 4. This sequence was double-checked several times. It could be shown that differences in the sequence of fpep3_1B-IR3seq compared to published sequence data could be explained by mutations already present in the starting constructs used for cloning fjun_1B-R408IR and fpep3_1B-IR3seq.

Co-transformation experiments (**Fig. 5**) using combinations of pIG10.3 or pOK1 phagemids (both with f1 oris) with fjun_1B ("wt" fd phage), fjun_1B-R408-IR (containing the DraIII/BsrGI fragment from R408) or fpep3_1B-IR3 (containing the DraIII/BsrGI fragment from R408 and the PCR mutation) revealed that the PCR mutation is not necessary for the IR phenotype, at least judged by the ability to be co-transformable with a phagemid and the ability of individual co-transformants to grow in liquid culture (cam/amp selection).

Additionally, the interacting protein partner p75ICD was cloned as a C-terminal fusion to the infectivity-mediating domains (N1-N2) of gIII (infectivity-mediating particle (IMP) fusion) resulting in constructs fIMPp75-IR3 and pIG10.3-IMPp75.

The IR phage was tested with the SIP pairing fpep3_1B-IR3seq3/ pIG10.3-IMPp75 (which gives a higher titer than fos/jun SIP) in the presence of the negative control combination fjun_1B-IR3seq3/ pIG10.3-IMPp75 (**Fig. 6**). A SIP hetero-polyphage titer of 1.5 x 10^5/ml (cam/amp-resistant transductants) was achieved with fpep3_1B-IR3seq3/ pIG10.3-IMPp75. To test SIP sensitivity in a model library vs. library setting, co-transformants of fpep3_1B-IR3seq3/ pIG10.3-IMPp75 were diluted in an excess fjun_1B-IR3/ pIG10.3-IMPp75 and the supernatant of the bacterial co-culture was assayed for SIP hetero-polyphages. This showed that down to a dilution of 10^{-5} to 10^{-6} can be recovered (**Fig. 7**).

To prove that only the correct phage vector is present in SIP polyphage transductants, DNA of positive (fpep3_1B-IR3seq3/ pIG10.3-IMPp75) and negative (fjun_1B-IR3/ pIG10.3-IMPp75) control co-transformants, as well as DNA from the SIP polyphage transductants derived from SIP phages produced by the mix of positive and negative control bacteria was analyzed by PCR (**Fig. 8**). Primers FR614 (5'-GCTCTAGATAACGAGGGC-3') and FR627 (5'-CGCAAGCTTAAGACTCCT-TATTACGC-3') amplify the phage region from the start of ompA to the end of gIII. PCR products derived from fpep3_1B-IR3seq3 and fjun_1B-IR3 can be discriminated by size. Gel analysis of the above samples verified that only the expected fpep3_1B-IR3seq3 phage was present in SIP polyphage transductants (6 analyzed).

To physically demonstrate the existence of hetero-polyphages (which have phage and phagemid co-packaged) when using the IR phage vector, phages produced by co-transformants of fIR3/pIG10.3-IMPp75 and as a control fjun_1B/JB61 ("wt" phage plus complementing gIII plasmid) were separated on an agarose gel (**Fig. 9**). This showed that the fIR3/pIG10.3-IMPp75 combination produced substantially more slower migrating (thus bigger) phages than the fjun_1B/JB61 control combination. The ratio was almost inversed. Elution of phages from various regions of the gel and subsequent titering of the eluate on plating cells showed that the upper gel region contained a significant portion of double resistance-transducing phages which thus can be regarded as hetero-polyphages.

The pairs fpep3_1B-IR3 and pIG10.3-IMPp75 as well as fIMPp75-IR3 and pIG10.3-pep10 were co-transformed into DH5α, individual cam/amp resistant clones were grown and the culture supernatant was tested on K91 cells for SIP phage production (**Fig. 10**). The combinations fpep3_1B-IR3/pIG10.3-IMPp75 and fIMPp75-IR3/pIG10.3-pep10 gave a titer of $1.5x10^5$ t.u./ml and $5x10^3$ t.u./ml, respectively when assayed for cam/amp-resistant transductants. The titer for each combination when assayed on LB cam was nearly the same as when assayed on LB cam/amp. This demonstrated efficient co-packaging of phage and phagemid DNA to almost 100 %, as seen before with the initial fjun_1B-R408IR and pOK1deltajun combination. To proof the existence of polyphages which individually co-transduce phage and phagemid DNA simultaneously, and to rule out the possibility of transduction of the two resistance markers by independent (and thus random) co-infection by two different phages which have only phage or phagemid packaged, a statistical test was performed. Defined, identical aliquots of bacterial culture supernatants of an individual co-transformant representing each of the two SIP vector combinations described above (fpep3_1B-IR3/pIG10.3-IMPp75 and fIMPp75-IR3/pIG10.3-pep10) were either used individually to infect K91 cells followed by selection on LB cam and LB amp plates, or the same supernatant aliquots from the two vector combinations were mixed before infection of K91 cells and selection on LB cam/amp. 117 cam-resistant, 328 amp-resistant and 141 cam/amp-resistant transforming units were present in the supernatant aliquot from the fIMPp75-IR3/pIG10.3-pep10 combination and 40 cam-resistant, 30 amp-resistant and 23 cam/amp-resistant transforming units were present in the supernatant aliquot from the fpep3_1B-IR3/pIG10.3-IMPp75 combination. The mix of both supernatant aliquots contained 166 cam-resistant and 162 cam/amp-resistant transforming units, exactly corresponding to the expected numbers which would be obtained by adding up the transducing units of the two individual aliquots. 48 cam/amp-resistant transductant colonies were picked from the plate were the mix of the two individual aliquots was used for infection and were analyzed by restriction digest. This showed that only the correct, SIP phage-producing vector combination (5 clones containing the fpep3_1B-IR3/pIG10.3-IMPp75 and 43 clones containing the fIMPp75-IR3/pIG10.3-pep10 combination; this represents a ratio of the two input vector combinations in the analyzed transductants of 1 : 8.6 (fpep3_1B-IR3/pIG10.3-IMPp75 : fIMPp75-IR3/pIG10.3-pep10), which is very similar to the 1 : 6.1 (fpep3_1B-IR3/pIG10.3-IMPp75 : fIMPp75-IR3/pIG10.3-pep10) ratio of double-resistant input phages in this experiment) occured in all analyzed

transductants, verifying the presence of hetero-polyphages by ruling out the possibility of random co-infection and thus incorrect, random combination by two out of four possible monophage and/or homo-polyphage populations (fpep3_1B-IR3, pIG10.3-IMPp75, fIMPp75-IR3 and pIG10.3-pep10) each containing only one type of vector (phage **or** phagemid). Statistically, co-infection of the same bacterium by two separate phages was practically already excluded by the small numbers of infective phages containing at least one resistance marker (166 cam-resistant and 358 amp-resistant phages) which were used in the above experiment. Co-infection of the same bacterium (of a total of 10^7 bacteria) by one of the 166 cam-resistant phages and one of the 358 amp-resistant phages has a probability of $6x10^{-10}$. Moreover, in this scenario incorrect combinations of individual phage and phagemid vectors (e.g. fpep3_1B-IR3/ pIG10.3-pep10 and fIMPp75-IR3/ pIG10.3-IMPp75) would be possible. The fact that only the correct vector combinations were found in all 48 transductants analyzed from this experiment further proved that co-transduction by hetero-polyphage and not random co-infection by homo-polyphage or monophage was the mechnism by which double-resistance was transduced.

2.3.: Construction of a phage-display system for Fab display

The constructs described in 3.2. can easily be modified to achieve the display of Fabs or a Fab library. In fpep3_1B-IR3seq, the jun part can be replaced by a VL-CL light chain repertoire having the appropriate 3'- and 5'-restriction sites similarly as described for pep_3-to construct fVL_1B-R408IR. In pIG10.3-IMPp75, the IMPp75 construct can be replaced by a repertoire of VH-CH1 heavy chains. After co-transformation of both repertoires into host cells and expression, a library of phage particles displaying Fab fragments is produced. Since fpep3_1B-IR3seq was set up for a SIP experiment by having just the C-terminal domain of gIII, the corresponding Fab-displaying phage particles are non-infectious. By adding a target molecule fused to an infectivity-mediating particle (N1-N2 domain of gIIIp), phages displaying target-binding Fab fragments can be selected by infecting host cells.

By replacing the truncated gIII part described above by a full-length copy of gIII, a Fab-display library of infectious phage particles is obtained, which can be screened against immobilized targets. Binding phages can be eluted and used to infect host cells.

By selecting for transductants conferring cam/amp-resistance to their host cells, polyphage infections can be selected in both cases. Thereby the information about both chains of the selected Fab fragments can be retrieved.

CLAIMS

1. A method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes,
said method being characterized by screening or selecting for polyphage particles that contain said combination.

2. The method of claim 1, comprising the steps of
(a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to, and displayed at, the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
(b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules of said second library are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
(c) optionally, providing nucleic acid sequences encoding further members of a multimeric (poly)peptide complex;
(d) expressing members of said libraries of recombinant vectors mentioned in steps (a), (b), and optionally nucleic acid sequences mentioned in step (c), in appropriate host cells under appropriate conditions, so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
(e) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
(f) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said

multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;

(g) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (f);

(h) identifying said combination of nucleic acid sequences.

3. The method of claim 1, comprising the steps of

(a) expressing in appropriate host cells under appropriate conditions

(aa) genetically diverse nucleic acid sequences contained in a first library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to and displayed at the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;

(ab) genetically diverse nucleic acid sequences contained in a second library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;

(ac) optionally, nucleic acid sequences encoding further members of a multimeric (poly)peptide complex,

so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;

(b) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;

(c) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;

(d) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (c);

(e) identifying said combination of nucleic acid sequences.

4. The method of anyone of claims 1 to 3, wherein the vectors of said first and said second library are a combination of a phage vector and a phagemid vector.

5. The method of anyone of claims 1 to 3, wherein the vectors of said first and said second library are a combination of two phagemid vectors, said appropriate conditions comprising complementation of phage genes by a helper phage.

6. The method of claim 5, wherein said two phagemid vectors are compatible.

7. The method of claim 6, wherein said two phagemid vectors comprise a ColE1 and a p15A plasmid origin of replication.

8. The method of claim 6, wherein said two phagemid vectors comprise a ColE1 and a mutated ColE1 origin.

9. The method of anyone of claims 4 to 8, wherein said vectors and/or said helper phage comprise different phage origins of replication.

10. The method of anyone of claim 4 to 9, wherein said phage vector, said phagemid vector(s) and/or said helper phage are interference resistant.

11. The method of claim 10, wherein said phage vector, said phagemid vector(s) and/or said helper phage have mutations in the phage intergenic region(s), preferably in positions corresponding to position 5986 of f1, and/or in gene II, preferably in positions corresponding to position 143 of f1.

12. The method of anyone of claims 10 to 11, wherein said phage vector, said phagemid vector(s) and/or said helper phage are, or are derived from, IR1 mutants such as R176, R382, R383, R407, R408, or from IR2 mutants.

13. The method of anyone of claims 4 to 11, wherein said vectors and/or said helper phage comprise hybrid nucleic acid sequences of f1, fd, and/or M13 derived sequences.

14. The method of anyone of claims 1 to 13, wherein said vector is, or is derived from, fpep3_1B-IR3seq with the sequence listed in Figure 4.

15. The method of claim 14, wherein said derivative is a phage comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

16. The method of claim 14, wherein said derivative is a phagemid comprising essentially the phage origin of replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

17. The method of claim 14, wherein said derivative is a helper phage comprising essentially the phage origin of replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

18. The method of anyone of claims 15 to 17, said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

19. The method of anyone of claims 1 to 18, wherein the gene VII contained in any of said vectors contains an amber mutation.

20. The method of claim 19, wherein said mutation is identical to those found in phage vectors R68 or R100.

21. The method of anyone of claims 1 to 20, wherein the gene IX contained in any of said vectors contains an amber mutation.

22. The method of claim 21, wherein said mutation is identical to that found in phage vector N18.

23. The method of anyone of claims 1 to 22, wherein said phage coat protein is gIIIp or gVIIIp.

24. The method of anyone of claims 1 to 23, wherein said phage particles are infectious by having a full-length copy of gIIIp.

25. The method of anyone of claims 1 to 24, wherein said phage particles are non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complexes with a corresponding partner coupled to an infectivity-mediating particle.

26. The method of claim 25, wherein said truncated gIIIp comprises the C-terminal domain of gIIIp.

27. The method of claim 26, wherein said truncated gIIIp is derived from phage fCA55.

28. The method of anyone of claims 1 to 27, wherein said predetermined property is binding to a target.

29. The method of claim 28, wherein said multimeric (poly)peptide complex is a fragment of an immunoglobulin superfamily member.

30. The method of claim 29, wherein said multimeric (poly)peptide complex is a fragment of an immunoglobulin.

31. The method of claim 30, wherein said fragment is an Fv, dsFv or Fab fragment.

32. The method of anyone of claims 1 to 27, wherein said predetermined property is the activity to perform or to catalyze a reaction.

33. The method of claim 32, wherein said multimeric (poly)peptide complex is an enzyme.

34. The method of claim 33, wherein said multimeric (poly)peptide complex is a fragment of a catalytic antibody.

35. The method of claim 34, wherein said fragment is an Fv, dsFv or Fab fragment.

36. The method of anyone of claims 1 to 35, wherein said selectable and/or screenable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

37. The method of anyone of claims 1 to 36, wherein said generation of said first and second screenable and/or selectable property is achieved after infection of appropriate host cells by said collection of phage particles.

38. The method of anyone of claims 1 to 37, wherein said identification of said nucleic acid sequences is effected by sequencing.

39. The method of anyone of claims 1 to 38, wherein said host cells are E.coli XL-1 Blue, K91 or derivatives thereof, TG1, XL1kann or TOP10F.

40. A polyphage particle which

(a) contains

(i) a first recombinant vector molecule that comprises a nucleic acid sequence, which encodes a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, and that carries or encodes a first selectable and/or screenable property, and

(ii) a second recombinant vector molecule that comprises a nucleic acid sequence, which encodes a second member of a multimeric (poly)peptide complex, and that

carries or encodes a second selectable and/or screenable property different from said first property;

and (b) displays said multimeric (poly)peptide complex at its surface.

41. The polyphage particle according to claim 40 wherein said phage coat protein is the gIIIp.

42. The polyphage particle according to claim 41 wherein said particles is infectious by having a full-length copy of gIIIp present, either in said fusion protein, or in an additional wild-type copy.

43. The polyphage particle according to claim 41 wherein said particles is non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complex with a corresponding partner coupled to an infectivity-mediating particle.

44. The phage vector fpep3_1B-IR3seq with the sequence listed in Figure 4.

45. A phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

46. A phagemid vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

47. A helper phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

48. A vector according to anyone of claims 45 to 47, wherein said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

49. The use according to any of the vectors of anyone of claims 44 to 48 in the generation of polyphage particles containing a combination of at least two different vectors.

50. The use according to claim 49, wherein said combination of different vectors comprises nucleic acid sequences encoding members of a multimeric (poly)peptide complex.

51. The use according to claim 50, wherein said combination of different vectors comprises nucleic acid sequences encoding interacting (poly)peptides/proteins.

Figure 1: General description of the polyphage principle

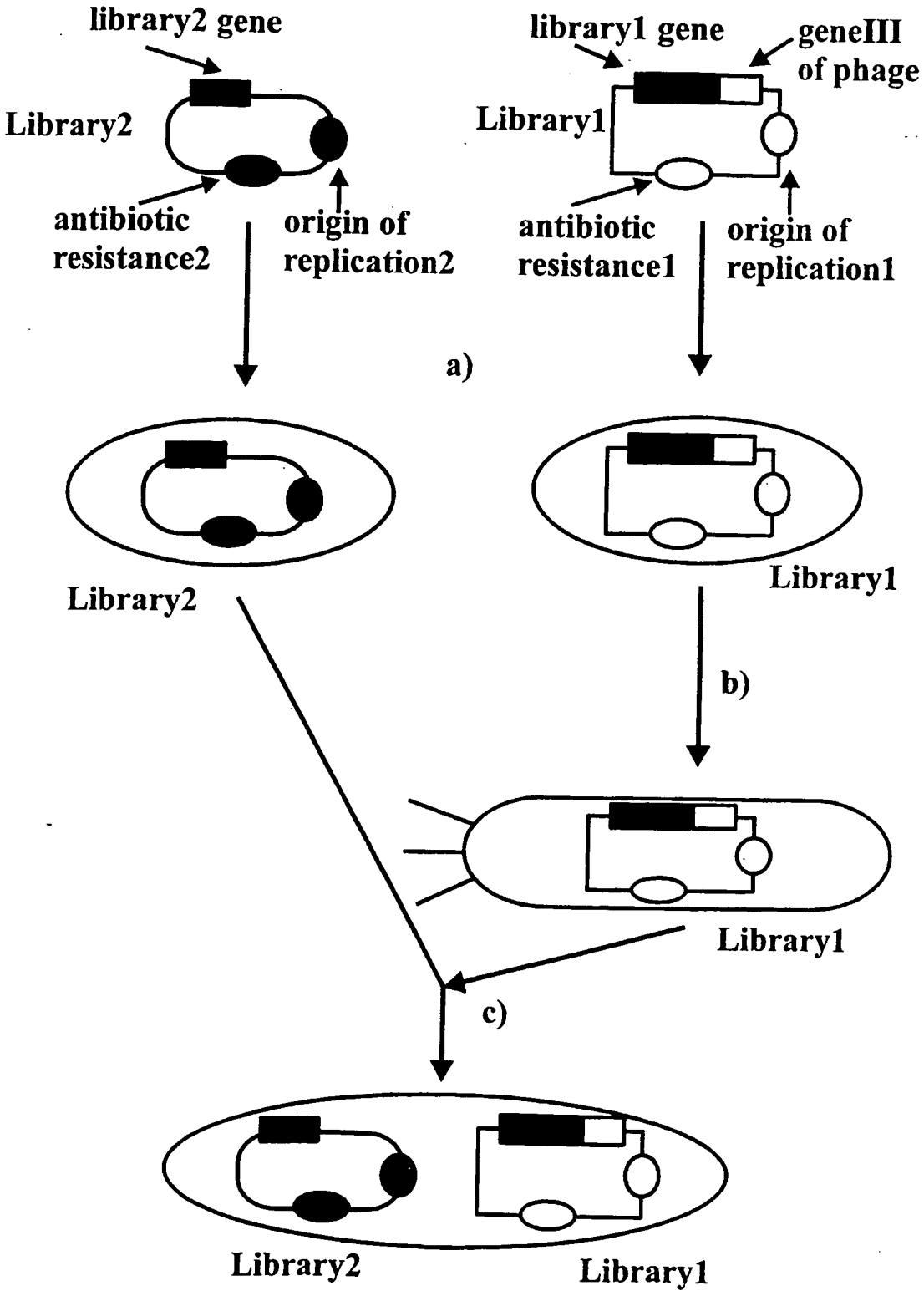


Figure 1: General description of the polyphage principle (cont.)

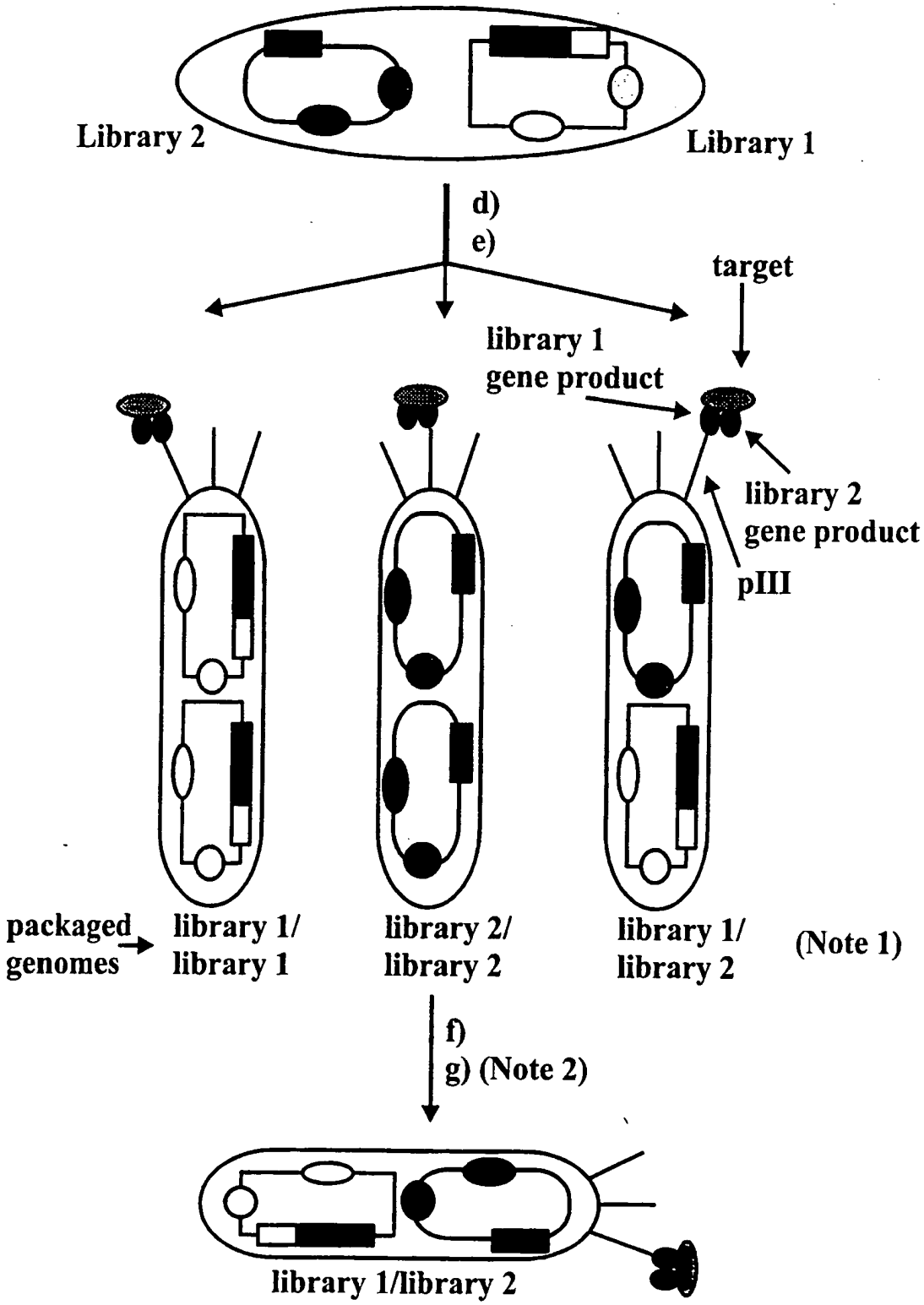
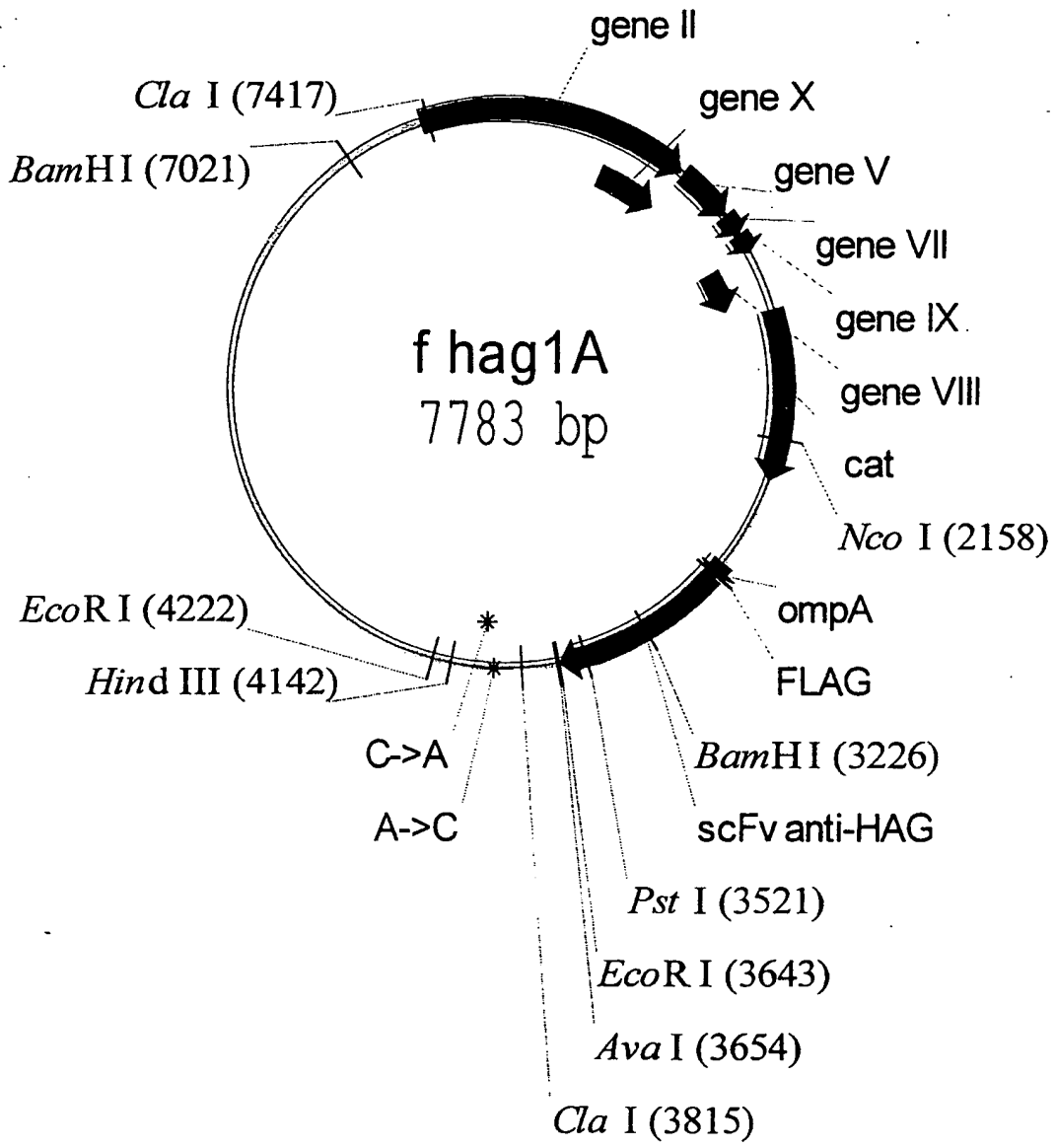


Figure 2


gene II
Cla I (7417)
gene X
BamHI (7021)
gene V
gene VII
gene IX
f hag1A
7783 bp
gene VIII
cat
Nco I (2158)
EcoR I (4222)
ompA
Hind III (4142)
FLAG
C->A
BamHI (3226)
A->C
scFv anti-HAG
Pst I (3521)
EcoR I (3643)
Ava I (3654)
Cla I (3815)

```
   1  AACGCTACTA CCATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC
      TTGCGATGAT GGTAATCATC TTAACTACGG TGGAAAAGTC GAGCGCGGGG

  51  AAATGAAAAT ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA
      TTTACTTTTA TATCGATTTG TCCAATAACT GGTAAACGCT TTACATAGAT

 101  ATGGTCAAAC TAAATCTACT CGTTCGCAGA ATTGGGAATC AACTGTTACA
      TACCAGTTTG ATTTAGATGA GCAAGCGTCT TAACCCTTAG TTGACAATGT

 151  TGGAATGAAA CTTCCAGACA CCGTACTTTA GTTGCATATT TAAAACATGT
      ACCTTACTTT GAAGGTCTGT GGCATGAAAT CAACGTATAA ATTTTGTACA

 201  TGAACTACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA TCCGCAAAAA
      ACTTGATGTC GTGGTCTAAG TCGTTAATTC GAGATTCGGT AGGCGTTTTT

 251  TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTGTCTAA TCCTGACCTG
      ACTGGAGAAT AGTTTTCCTC GTTAATTTCC ATGACAGATT AGGACTGGAC

 301  TTGGAATTTG CTTCCGGTCT GGTTCGCTTT GAGGCTCGAA TTGAAACGCG
      AACCTTAAAC GAAGGCCAGA CCAAGCGAAA CTCCGAGCTT AACTTTGCGC

 351  ATATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATTCGCT
      TATAAACTTC AGAAAGCCCG AAGGAGAATT AGAAAAACTA CGTTAAGCGA

 401  TTGCTTCTGA CTATAATAGA CAGGGTAAAG ACCTGATTTT TGATTTATGG
      AACGAAGACT GATATTATCT GTCCCATTTC TGGACTAAAA ACTAAATACC

 451  TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA
      AGTAAGAGCA AAAGACTTGA CAAATTTCGT AAACTCCCCC TAAGTTACTT

 501  TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
      ATAAATACTG CTAAGGCGTC ATAACCTGCG ATAGGTCAGA TTTGTAAAAT

 551  CAATTACCCC CTCTGGCAAA ACTTCCTTTG CAAAAGCCTC TCGCTATTTT
      GTTAATGGGG GAGACCGTTT TGAAGGAAAC GTTTTCGGAG AGCGATAAAA

 601  GGTTTCTATC GTCGTCTGGT TAATGAGGGT TATGATAGTG TTGCTCTTAC
      CCAAAGATAG CAGCAGACCA ATTACTCCCA ATACTATCAC AACGAGAATG

 651  CATGCCTCGT AATTCCTTTT GGCGTTATGT ATCTGCATTA GTTGAGTGTG
      GTACGGAGCA TTAAGGAAAA CCGCAATACA TAGACGTAAT CAACTCACAC

 701  GTATTCCTAA ATCTCAATTG ATGAATCTTT CCACCTGTAA TAATGTTGTT
      CATAAGGATT TAGAGTTAAC TACTTAGAAA GGTGGACATT ATTACAACAA

 751  CCGTTAGTTC GTTTTATTAA CGTAGATTTT TCCTCCCAAC GTCCTGACTG
      GGCAATCAAG CAAAATAATT GCATCTAAAA AGGAGGGTTG CAGGACTGAC

 801  GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA AAATGATTAA
      CATATTACTC GGTCAAGAAT TTTAGCGTAT TCCATTAAGT TTTACTAATT
```

```
851  AGTTGAAATT AAACCGTCTC AAGCGCAATT TACTACCCGT TCTGGTGTTT
     TCAACTTTAA TTTGGCAGAG TTCGCGTTAA ATGATGGGCA AGACCACAAA

901  CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTTG TTACGTTGAT
     GAGCAGTCCC GTTCGGAATA AGTGACTTAC TCGTCGAAAC AATGCAACTA

951  TTGGGTAATG AATATCCGGT GCTTGTCAAG ATTACTCTCG ACGAAGGTCA
     AACCCATTAC TTATAGGCCA CGAACAGTTC TAATGAGAGC TGCTTCCAGT

1001 GCCAGCGTAT GCGCCTGGTC TGTACACCGT GCATCTGTCC TCGTTCAAAG
     CGGTCGCATA CGCGGACCAG ACATGTGGCA CGTAGACAGG AGCAAGTTTC

1051 TTGGTCAGTT CGGTTCTCTT ATGATTGACC GTCTGCGCCT CGTTCCGGCT
     AACCAGTCAA GCCAAGAGAA TACTAACTGG CAGACGCGGA GCAAGGCCGA

1101 AAGTAACATG GAGCAGGTCG CGGATTTCGA CACAATTTAT CAGGCGATGA
     TTCATTGTAC CTCGTCCAGC GCCTAAAGCT GTGTTAAATA GTCCGCTACT

1151 TACAAATCTC CGTTGTACTT TGTTTCGCGC TTGGTATAAT CGCTGGGGGT
     ATGTTTAGAG GCAACATGAA ACAAAGCGCG AACCATATTA GCGACCCCCA

1201 CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTTAGGTTGG
     GTTTCTACTC ACAAAATCAC ATAAGAAAGC GGAGAAAGCA AAATCCAACC

1251 TGCCTTCGTA GTGGCATTAC GTATTTTACC CGTTTAATGG AAACTTCCTC
     ACGGAAGCAT CACCGTAATG CATAAAATGG GCAAATTACC TTTGAAGGAG

1301 ATGCGTAAGT CTTTAGTCCT CAAAGCCTCC GTAGCCGTTG CTACCCTCGT
     TACGCATTCA GAAATCAGGA GTTTCGGAGG CATCGGCAAC GATGGGAGCA

1351 TCCGATGCTG TCTTTCGCTG CTGAGGGTGA CGATCCCGCA AAAGCGGCCT
     AGGCTACGAC AGAAAGCGAC GACTCCCACT GCTAGGGCGT TTTCGCCGGA

1401 TTGACTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA TGCGTGGGCG
     AACTGAGGGA CGTTCGGAGT CGCTGGCTTA TATAGCCAAT ACGCACCCGC

1451 ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTTAAGAA
     TACCAACAAC AGTAACAGCC GCGTTGATAG CCATAGTTCG ACAAATTCTT

1501 ATTCACCTCG AAAGCAAGCT GATAAAGGAG GTTTCTCGAT CGAGACGTTN
     TAAGTGGAGC TTTCGTTCGA CTATTTCCTC CAAAGAGCTA GCTCTGCAAN

1551 NNNGAGGTTC CAACTTTCAC CATAATGAAA TAAGATCACT ACCGGGCGTA
     NNNCTCCAAG GTTGAAAGTG GTATTACTTT ATTCTAGTGA TGGCCCGCAT

1601 TTTTTTGAGT TATCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA
     AAAAAACTCA ATAGCTCTAA AAGTCCTCGA TTCCTTCGAT TTTACCTCTT

1651 AAAAATCACT GGATATACCA CCGTTGATAT ATCCCAATGG CATCGTAAAG
     TTTTTAGTGA CCTATATGGT GGCAACTATA TAGGGTTACC GTAGCATTTC
```

```
1701  AACATTTTGA GGCATTTCAG TCAGTTGCTC AATGTACCTA TAACCAGACC
      TTGTAAAACT CCGTAAAGTC AGTCAACGAG TTACATGGAT ATTGGTCTGG

1751  GTTCAGCTGG ATATTACGGC CTTTTTAAAG ACCGTAAAGA AAAATAAGCA
      CAAGTCGACC TATAATGCCG GAAAAATTTC TGGCATTTCT TTTTATTCGT

1801  CAAGTTTTAT CCGGCCTTTA TTCACATTCT TGCCCGCCTG ATGAATGCTC
      GTTCAAAATA GGCCGGAAAT AAGTGTAAGA ACGGGCGGAC TACTTACGAG

1851  ATCCGGAGTT CCGTATGGCA ATGAAAGACG GTGAGCTGGT GATATGGGAT
      TAGGCCTCAA GGCATACCGT TACTTTCTGC CACTCGACCA CTATACCCTA

1901  AGTGTTCACC CTTGTTACAC CGTTTTCCAT GAGCAAACTG AAACGTTTTC
      TCACAAGTGG GAACAATGTG GCAAAAGGTA CTCGTTTGAC TTTGCAAAAG

1951  ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT
      TAGCGAGACC TCACTTATGG TGCTGCTAAA GGCCGTCAAA GATGTGTATA

2001  ATTCGCAAGA TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA
      TAAGCGTTCT ACACCGCACA ATGCCACTTT TGGACCGGAT AAAGGGATTT

2051  GGGTTTATTG AGAATATGTT TTTCGTCTCA GCCAATCCCT GGGTGAGTTT
      CCCAAATAAC TCTTATACAA AAAGCAGAGT CGGTTAGGGA CCCACTCAAA

2101  CACCAGTTTT GATTTAAACG TGGCCAATAT GGACAACTTC TTCGCCCCCG
      GTGGTCAAAA CTAAATTTGC ACCGGTTATA CCTGTTGAAG AAGCGGGGGC

            NcoI
           -------
2151  TTTTCACCAT GGGCAAATAT TATACGCAAG GCGACAAGGT GCTGATGCCG
      AAAAGTGGTA CCCGTTTATA ATATGCGTTC CGCTGTTCCA CGACTACGGC

2201  CTGGCGATTC AGGTTCATCA TGCCGTCTGT GATGGCTTCC ATGTCGGCAG
      GACCGCTAAG TCCAAGTAGT ACGGCAGACA CTACCGAAGG TACAGCCGTC

2251  AATGCTTAAT GAATTACAAC AGTACTGCGA TGAGTGGCAG GGCGGGGCGT
      TTACGAATTA CTTAATGTTG TCATGACGCT ACTCACCGTC CCGCCCCGCA

2301  AATTTTTTTA AGGCAGTTAT TGGTGCCCTT AAACGCCTGG TGCTACGCCT
      TTAAAAAAAT TCCGTCAATA ACCACGGGAA TTTGCGGACC ACGATGCGGA

2351  GAATAAGTGA TAATAAGCGG ATGAATGGCA GAAATTCGAA AGCAAATTCG
      CTTATTCACT ATTATTCGCC TACTTACCGT CTTTAAGCTT TCGTTTAAGC

2401  ACCCGGTCGT CGGTTCAGGG CAGGGTCGTT AAATAGCCGC TTATGTCTAT
      TGGGCCAGCA GCCAAGTCCC GTCCCAGCAA TTTATCGGCG AATACAGATA

2451  TGCTGGTTTA CCGGTTTATT GACTACCGGA AGCAGTGTGA CCGTGTGCTT
      ACGACCAAAT GGCCAAATAA CTGATGGCCT TCGTCACACT GGCACACGAA

2501  CTCAAATGCC TGAGGCCAGT TTGCTCAGGC TCTCCCCGTG GAGGTAATAA
      GAGTTTACGG ACTCCGGTCA AACGAGTCCG AGAGGGGCAC CTCCATTATT
```

```
2551  TTGCTCGACC GATAAAAGCG GCTTCCTGAC AGGAGGCCGT TTTGTTTTGC
      AACGAGCTGG CTATTTTCGC CGAAGGACTG TCCTCCGGCA AAACAAAACG

2601  AGCCCACCTC AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC
      TCGGGTGGAG TTGCGTTAAT TACACTCAAT CGAGTGAGTA ATCCGTGGGG

2651  AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC
      TCCGAAATGT GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG

2701  GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTTCT
      CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAAAGA

2751  AGATAACGAG GGCAAATCAT GAAAAAGACA GCTATCGCGA TTGCAGTGGC
      TCTATTGCTC CCGTTTAGTA CTTTTTCTGT CGATAGCGCT AACGTCACCG

2801  ACTGGCTGGT TTCGCTACCG TAGCGCAGGC CGACTACAAA GATATCGTTA
      TGACCGACCA AAGCGATGGC ATCGCGTCCG GCTGATGTTT CTATAGCAAT

2851  TGACCCAGTC ACCGTCCTCC CTGACCGTTA CCGCTGGTGA AAAAGTTACC
      ACTGGGTCAG TGGCAGGAGG GACTGGCAAT GGCGACCACT TTTTCAATGG

2901  ATGTCCTGCA CCTCCTCCCA GTCCCTGTTC AACTCCGGTA AACAGAAAAA
      TACAGGACGT GGAGGAGGGT CAGGGACAAG TTGAGGCCAT TTGTCTTTTT

2951  CTACCTGACC TGGTATCAGC AGAAACCGGG TCAGCCACCG AAAGTTCTGA
      GATGGACTGG ACCATAGTCG TCTTTGGCCC AGTCGGTGGC TTTCAAGACT

3001  TCTACTGGGC TTCCACCCGT GAATCCGGTG TTCCAGACCG TTTCACCGGT
      AGATGACCCG AAGGTGGGCA CTTAGGCCAC AAGGTCTGGC AAAGTGGCCA

3051  TCCGGTTCCG GCACCGACTT CACCCTGACC ATCTCCTCCG TTCAGGCTGA
      AGGCCAAGGC CGTGGCTGAA GTGGGACTGG TAGAGGAGGC AAGTCCGACT

3101  AGACCTGGCT GTTTACTACT GCCAGAACGA CTACTCCAAC CCACTGACCT
      TCTGGACCGA CAAATGATGA CGGTCTTGCT GATGAGGTTG GGTGACTGGA

3151  TCGGTGGTGG CACCAAACTG GAACTTAAGC GCGCTGGTGG TGGAGGGTCT
      AGCCACCACC GTGGTTTGAC CTTGAATTCG CGCGACCACC ACCTCCCAGA

                                  BamHI
                                  ~~~~~~
3201  GGAGGAGGTG GGAGTGGGGG AGGTGGATCC GGCGGGGGAG GTTCAGGGGG
      CCTCCTCCAC CCTCACCCCC TCCACCTAGG CCGCCCCCTC CAAGTCCCCC

3251  TGGCGGTAGT GGAGGGGGCG GTTCAGAAGT TCAACTAGTT GAATCCGGTG
      ACCGCCATCA CCTCCCCCGC CAAGTCTTCA AGTTGATCAA CTTAGGCCAC

3301  GTGACCTGGT TAAACCGGGT GGTTCCCTGA AACTGTCCTG CGCTGCTTCC
      CACTGGACCA ATTTGGCCCA CCAAGGGACT TTGACAGGAC GCGACGAAGG
```

```
3351  GGTTTCTCCT TCTCCTCCTA CGGTATGTCC TGGGTTCGTC AGACCCCGGA
      CCAAAGAGGA AGAGGAGGAT GCCATACAGG ACCCAAGCAG TCTGGGGCCT

3401  CAAACGTCTG GAATGGGTTG CTACCATCTC CAACGGTGGT GGTTACACCT
      GTTTGCAGAC CTTACCCAAC GATGGTAGAG GTTGCCACCA CCAATGTGGA

3451  ACTACCCGGA CTCCGTTAAA GGTCGTTTCA CCATCTCCCG TGACAACGCT
      TGATGGGCCT GAGGCAATTT CCAGCAAAGT GGTAGAGGGC ACTGTTGCGA

                        PstI
                       -------
3501  AAAAACACCC TGTACCTGCA GATGTCCTCC CTGAAATCCG AAGACTCAGC
      TTTTTGTGGG ACATGGACGT CTACAGGAGG GACTTTAGGC TTCTGAGTCG

3551  TATGTACTAC TGCGCTCGTC GTGAACGTTA CGACGAAAAC GGTTTCGCTT
      ATACATGATG ACGCGAGCAG CACTTGCAAT GCTGCTTTTG CCAAAGCGAA

                                                      EcoRI
                                                     ------
3601  ACTGGGGTCA GGGTACCCTG GTTACCGTTT CAGCTTCCGG AGAATTCGAG
      TGACCCCAGT CCCATGGGAC CAATGGCAAA GTCGAAGGCC TCTTAAGCTC

        AvaI
       ------
3651  GCCTCGGGGG CCGAGGGCGG CGGTTCTGGT TCCGGTGATT TTGATTATGA
      CGGAGCCCCC GGCTCCCGCC GCCAAGACCA AGGCCACTAA AACTAATACT

3701  AAAAATGGCA AACGCTAATA AGGGGGCTAT GACCGAAAAT GCCGATGAAA
      TTTTTACCGT TTGCGATTAT TCCCCCGATA CTGGCTTTTA CGGCTACTTT

3751  ACGCGCTACA GTCTGACGCT AAAGGCAAAC TTGATTCTGT CGCTACTGAT
      TGCGCGATGT CAGACTGCGA TTTCCGTTTG AACTAAGACA GCGATGACTA

                    ClaI
                   ------
3801  TACGGTGCTG CTATCGATGG TTTCATTGGT GACGTTTCCG GCCTTGCTAA
      ATGCCACGAC GATAGCTACC AAAGTAACCA CTGCAAAGGC CGGAACGATT

3851  TGGTAATGGT GCTACTGGTG ATTTTGCTGG CTCTAATTCC CAAATGGCTC
      ACCATTACCA CGATGACCAC TAAAACGACC GAGATTAAGG GTTTACCGAG

3901  AAGTCGGTGA CGGTGATAAT TCACCTTTAA TGAATAATTT CCGTCAATAT
      TTCAGCCACT GCCACTATTA AGTGGAAATT ACTTATTAAA GGCAGTTATA

3951  TTACCTTCCC TCCCTCAATC GGTTGAATGT CGCCCTTTTG TCTTTGGCGC
      AATGGAAGGG AGGGAGTTAG CCAACTTACA GCGGGAAAAC AGAAACCGCG

4001  TGGTAAACCA TATGAATTTT CTATTGATTG TGACAAAATA AACTTATTCC
      ACCATTTGGT ATACTTAAAA GATAACTAAC ACTGTTTTAT TTGAATAAGG

4051  GTGGTGTCTT TGCGTTTCTT TTATATGTTG CCACCTTTAT GTATGTATTT
      CACCACAGAA ACGCAAAGAA AATATACAAC GGTGGAAATA CATACATAAA
```

HindIII
~~~~~~

4101 TCTACGTTTG CTAACATACT GCGTAATAAG GAGTCTTGAT AAGCTTCGAG
AGATGCAAAC GATTGTATGA CGCATTATTC CTCAGAACTA TTCGAAGCTC

4151 AAATTCACCT CGAAAGCAAG CTGATAAACC GATACAATTA AAGGCTCCTT
TTTAAGTGGA GCTTTCGTTC GACTATTTGG CTATGTTAAT TTCCGAGGAA

EcoRI
~~~~~~

4201 TTGGAGCCTT TTTTTTTGGA GAATTCAATC ATGCCAGTTC TTTTGGGTAT
AACCTCGGAA AAAAAAACCT CTTAAGTTAG TACGGTCAAG AAAACCCATA

4251 TCCGTTATTA TTGCGTTTCC TCGGTTTCCT TCTGGTAACT TTGTTCGGCT
AGGCAATAAT AACGCAAAGG AGCCAAAGGA AGACCATTGA AACAAGCCGA

4301 ATCTGCTTAC TTTCCTTAAA AAGGGCTTCG GTAAGATAGC TATTGCTATT
TAGACGAATG AAAGGAATTT TTCCCGAAGC CATTCTATCG ATAACGATAA

4351 TCATTGTTTC TTGCTCTTAT TATTGGGCTT AACTCAATTC TTGTGGGTTA
AGTAACAAAG AACGAGAATA ATAACCCGAA TTGAGTTAAG AACACCCAAT

4401 TCTCTCTGAT ATTAGCGCAC AATTACCCTC TGATTTTGTT CAGGGCGTTC
AGAGAGACTA TAATCGCGTG TTAATGGGAG ACTAAAACAA GTCCCGCAAG

4451 AGTTAATTCT CCCGTCTAAT GCGCTTCCCT GTTTTTATGT TATTCTCTCT
TCAATTAAGA GGGCAGATTA CGCGAAGGGA CAAAAATACA ATAAGAGAGA

4501 GTAAAGGCTG CTATTTTCAT TTTTGACGTT AAACAAAAAA TCGTTTCTTA
CATTTCCGAC GATAAAAGTA AAAACTGCAA TTTGTTTTTT AGCAAAGAAT

4551 TTTGGATTGG GATAAATAAA TATGGCTGTT TATTTTGTAA CTGGCAAATT
AAACCTAACC CTATTTATTT ATACCGACAA ATAAAACATT GACCGTTTAA

4601 AGGCTCTGGA AAGACGCTCG TTAGCGTTGG TAAGATTCAG GATAAAATTG
TCCGAGACCT TTCTGCGAGC AATCGCAACC ATTCTAAGTC CTATTTTAAC

4651 TAGCTGGGTG CAAAATAGCA ACTAATCTTG ATTTAAGGCT TCAAAACCTC
ATCGACCCAC GTTTTATCGT TGATTAGAAC TAAATTCCGA AGTTTTGGAG

4701 CCGCAAGTCG GGAGGTTCGC TAAAACGCCT CGCGTTCTTA GAATACCGGA
GGCGTTCAGC CCTCCAAGCG ATTTTGCGGA GCGCAAGAAT CTTATGGCCT

4751 TAAGCCTTCT ATTTCTGATT TGCTTGCTAT TGGTCGTGGT AATGATTCCT
ATTCGGAAGA TAAAGACTAA ACGAACGATA ACCAGCACCA TTACTAAGGA

4801 ACGACGAAAA TAAAAACGGT TTGCTTGTTC TTGATGAATG CGGTACTTGG
TGCTGCTTTT ATTTTTGCCA AACGAACAAG AACTACTTAC GCCATGAACC

4851 TTTAATACCC GTTCATGGAA TGACAAGGAA AGACAGCCGA TTATTGATTG
AAATTATGGG CAAGTACCTT ACTGTTCCTT TCTGTCGGCT AATAACTAAC

```
4901  GTTTCTTCAT GCTCGTAAAT TGGGATGGGA TATTATTTTT CTTGTTCAGG
      CAAAGAAGTA CGAGCATTTA ACCCTACCCT ATAATAAAAA GAACAAGTCC

4951  ATTTATCTAT TGTTGATAAA CAGGCGCGTT CTGCATTAGC TGAACACGTT
      TAAATAGATA ACAACTATTT GTCCGCGCAA GACGTAATCG ACTTGTGCAA

5001  GTTTATTGTC GCCGTCTGGA CAGAATTACT TTACCCTTTG TCGGCACTTT
      CAAATAACAG CGGCAGACCT GTCTTAATGA AATGGGAAAC AGCCGTGAAA

5051  ATATTCTCTT GTTACTGGCT CAAAAATGCC TCTGCCTAAA TTACATGTTG
      TATAAGAGAA CAATGACCGA GTTTTTACGG AGACGGATTT AATGTACAAC

5101  GTGTTGTTAA ATATGGTGAT TCTCAATTAA GCCCTACTGT TGAGCGTTGG
      CACAACAATT TATACCACTA AGAGTTAATT CGGGATGACA ACTCGCAACC

5151  CTTTATACTG GTAAGAATTT ATATAACGCA TATGACACTA AACAGGCTTT
      GAAATATGAC CATTCTTAAA TATATTGCGT ATACTGTGAT TTGTCCGAAA

5201  TTCCAGTAAT TATGATTCAG GTGTTTATTC ATATTTAACC CCTTATTTAT
      AAGGTCATTA ATACTAAGTC CACAAATAAG TATAAATTGG GGAATAAATA

5251  CACACGGTCG GTATTTCAAA CCATTAAATT TAGGTCAGAA GATGAAATTA
      GTGTGCCAGC CATAAAGTTT GGTAATTTAA ATCCAGTCTT CTACTTTAAT

5301  ACTAAAATAT ATTTGAAAAA GTTTTCTCGC GTTCTTTGTC TTGCGATAGG
      TGATTTTATA TAAACTTTTT CAAAAGAGCG CAAGAAACAG AACGCTATCC

5351  ATTTGCATCA GCATTTACAT ATAGTTATAT AACCCAACCT AAGCCGGAGG
      TAAACGTAGT CGTAAATGTA TATCAATATA TTGGGTTGGA TTCGGCCTCC

5401  TTAAAAAGGT AGTCTCTCAG ACCTATGATT TTGATAAATT CACTATTGAC
      AATTTTTCCA TCAGAGAGTC TGGATACTAA AACTATTTAA GTGATAACTG

5451  TCTTCTCAGC GTCTTAATCT AAGCTATCGC TATGTTTTCA AGGATTCTAA
      AGAAGAGTCG CAGAATTAGA TTCGATAGCG ATACAAAAGT TCCTAAGATT

5501  GGGAAAATTA ATTAATAGCG ACGATTTACA GAAGCAAGGT TATTCCATCA
      CCCTTTTAAT TAATTATCGC TGCTAAATGT CTTCGTTCCA ATAAGGTAGT

5551  CATATATTGA TTTATGTACT GTTTCAATTA AAAAAGGTAA TTCAAATGAA
      GTATATAACT AAATACATGA CAAAGTTAAT TTTTTCCATT AAGTTTACTT

5601  ATTGTTAAAT GTAATTAATT TTGTTTTCTT GATGTTTGTT TCATCATCTT
      TAACAATTTA CATTAATTAA AACAAAAGAA CTACAAACAA AGTAGTAGAA

5651  CTTTTGCTCA AGTAATTGAA ATGAATAATT CGCCTCTGCG CGATTTCGTG
      GAAAACGAGT TCATTAACTT TACTTATTAA GCGGAGACGC GCTAAAGCAC

5701  ACTTGGTATT CAAAGCAAAC AGGTGAATCT GTTATTGTCT CACCTGATGT
      TGAACCATAA GTTTCGTTTG TCCACTTAGA CAATAACAGA GTGGACTACA
```

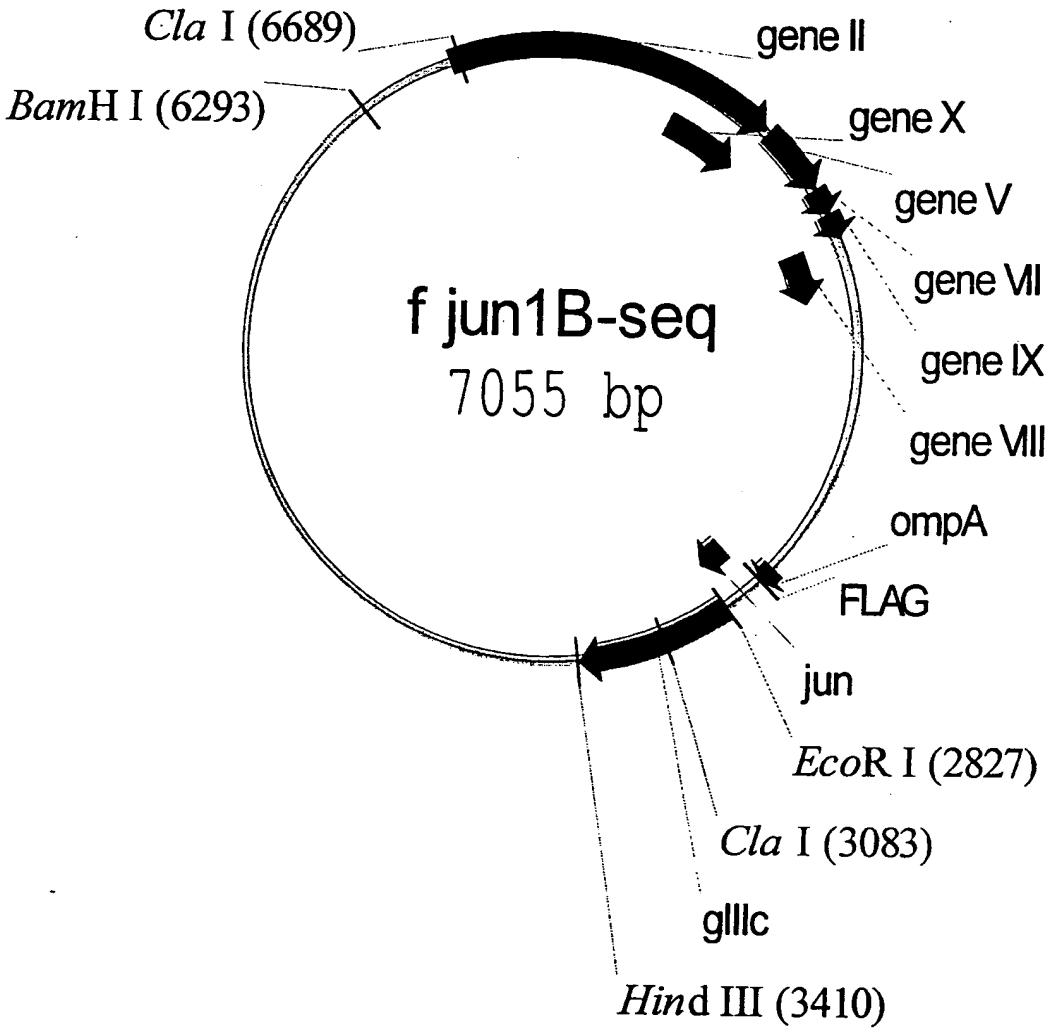
```
5751  TAAAGGTACA GTGACTGTAT ATTCCTCTGA CGTTAAGCCT GAAAATTTAC
      ATTTCCATGT CACTGACATA TAAGGAGACT GCAATTCGGA CTTTTAAATG

5801  GCAATTTCTT TATCTCTGTT TTACGTGCTA ATAATTTTGA TATGGTTGGC
      CGTTAAAGAA ATAGAGACAA AATGCACGAT TATTAAAACT ATACCAACCG

5851  TCAATTCCTT CCATAATTCA GAAATATAAC CCAAATAGTC AGGATTATAT
      AGTTAAGGAA GGTATTAAGT CTTTATATTG GGTTTATCAG TCCTAATATA

5901  TGATGAATTG CCATCATCTG ATATTCAGGA ATATGATGAT AATTCCGCTC
      ACTACTTAAC GGTAGTAGAC TATAAGTCCT TATACTACTA TTAAGGCGAG

5951  CTTCTGGTGG TTTCTTTGTT CCGCAAAATG ATAATGTTAC TCAAACATTT
      GAAGACCACC AAAGAAACAA GGCGTTTTAC TATTACAATG AGTTTGTAAA

6001  AAAATTAATA ACGTTCGCGC AAAGGATTTA ATAAGGGTTG TAGAATTGTT
      TTTTAATTAT TGCAAGCGCG TTTCCTAAAT TATTCCCAAC ATCTTAACAA

6051  TGTTAAATCT AATACATCTA AATCCTCAAA TGTATTATCT GTTGATGGTT
      ACAATTTAGA TTATGTAGAT TTAGGAGTTT ACATAATAGA CAACTACCAA

6101  CTAACTTATT AGTAGTTAGC GCCCCTAAAG ATATTTTAGA TAACCTTCCG
      GATTGAATAA TCATCAATCG CGGGGATTTC TATAAAATCT ATTGGAAGGC

6151  CAATTTCTTT CTACTGTTGA TTTGCCAACT GACCAGATAT TGATTGAAGG
      GTTAAAGAAA GATGACAACT AAACGGTTGA CTGGTCTATA ACTAACTTCC

6201  ATTAATTTTC GAGGTTCAGC AAGGTGATGC TTTAGATTTT TCCTTTGCTG
      TAATTAAAAG CTCCAAGTCG TTCCACTACG AAATCTAAAA AGGAAACGAC

6251  CTGGCTCTCA GCGCGGCACT GTTGCTGGTG GTGTTAATAC TGACCGTCTA
      GACCGAGAGT CGCGCCGTGA CAACGACCAC CACAATTATG ACTGGCAGAT

6301  ACCTCTGTTT TATCTTCTGC GGGTGGTTCG TTCGGTATTT TTAACGGCGA
      TGGAGACAAA ATAGAAGACG CCCACCAAGC AAGCCATAAA AATTGCCGCT

6351  TGTTTTAGGG CTATCAGTTC GCGCATTAAA GACTAATAGC CATTCAAAAA
      ACAAAATCCC GATAGTCAAG CGCGTAATTT CTGATTATCG GTAAGTTTTT

6401  TATTGTCTGT GCCTCGTATT CTTACGCTTT CAGGTCAGAA GGGTTCTATT
      ATAACAGACA CGGAGCATAA GAATGCGAAA GTCCAGTCTT CCCAAGATAA

6451  TCTGTTGGCC AGAATGTCCC TTTTATTACT GGTCGTGTAA CTGGTGAATC
      AGACAACCGG TCTTACAGGG AAAATAATGA CCAGCACATT GACCACTTAG

6501  TGCCAATGTA AATAATCCAT TTCAGACGGT TGAGCGTCAA AATGTTGGTA
      ACGGTTACAT TTATTAGGTA AAGTCTGCCA ACTCGCAGTT TTACAACCAT

6551  TTTCTATGAG TGTTTTTCCC GTTGCAATGG CTGGCGGTAA TATTGTTTTA
      AAAGATACTC ACAAAAAGGG CAACGTTACC GACCGCCATT ATAACAAAAT
```

```
6601  GATATAACCA GTAAGGCCGA TAGTTTGAGT TCTTCTACTC AGGCAAGTGA
      CTATATTGGT CATTCCGGCT ATCAAACTCA AGAAGATGAG TCCGTTCACT

6651  TGTTATTACT AATCAAAGAA GTATTGCGAC AACGGTTAAT TTGCGTGATG
      ACAATAATGA TTAGTTTCTT CATAACGCTG TTGCCAATTA AACGCACTAC

6701  GTCAGACTCT TTTGCTCGGT GGCCTCACTG ATTACAAAAA CACTTCTCAA
      CAGTCTGAGA AAACGAGCCA CCGGAGTGAC TAATGTTTTT GTGAAGAGTT

6751  GATTCTGGTG TGCCGTTCCT GTCTAAAATC CCTTTAATCG GCCTCCTGTT
      CTAAGACCAC ACGGCAAGGA CAGATTTTAG GGAAATTAGC CGGAGGACAA

6801  TAGCTCCCGT TCTGATTCTA ACGAGGAAAG CACGTTGTAC GTGCTCGTCA
      ATCGAGGGCA AGACTAAGAT TGCTCCTTTC GTGCAACATG CACGAGCAGT

6851  AAGCAACCAT AGTACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT
      TTCGTTGGTA TCATGCGCGG GACATCGCCG CGTAATTCGC GCCGCCCACA

6901  GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG
      CCACCAATGC GCGTCGCACT GGCGATGTGA ACGGTCGCGG GATCGCGGGC

6951  CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCTC CGGCTTTCCC
      GAGGAAAGCG AAAGAAGGGA AGGAAAGAGC GGTGCAAGAG GCCGAAAGGG

                          BamHI
                          ~~~~~~~
7001  CGTCAAGCTC TAAATCGGGG GATCCCTTTA GGGTTCCGAT TTAGTGCTTT
      GCAGTTCGAG ATTTAGCCCC CTAGGGAAAT CCCAAGGCTA AATCACGAAA

7051  ACGGCACCTC GACCTCCAAA AACTTGATTT GGGTGATGGT TCACGTAGTG
      TGCCGTGGAG CTGGAGGTTT TTGAACTAAA CCCACTACCA AGTGCATCAC

7101  GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG
      CCGGTAGCGG GACTATCTGC CAAAAAGCGG GAAACTGCAA CCTCAGGTGC

7151  TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC TCACAACTAA
      AAGAAATTAT CACCTGAGAA CAAGGTTTGA CCTTGTTGTG AGTGTTGATT

7201  CTCGGCCTAT TCTTTTGATT TATAAGGATT TTTGTCATTT TCTGCTTACT
      GAGCCGGATA AGAAAACTAA ATATTCCTAA AAACAGTAAA AGACGAATGA

7251  GGTTAAAAAA TAAGCTGATT TAACAAATAT TTAACGCGAA ATTTAACAAA
      CCAATTTTTT ATTCGACTAA ATTGTTTATA AATTGCGCTT TAAATTGTTT

7301  ACATTAACGT TTACAATTTA AATATTTGCT TATACAATCA TCCTGTTTTT
      TGTAATTGCA AATGTTAAAT TTATAAACGA ATATGTTAGT AGGACAAAAA

7351  GGGGCTTTTC TGATTATCAA CCGGGGTACA TATGATTGAC ATGCTAGTTT
      CCCCGAAAAG ACTAATAGTT GGCCCCATGT ATACTAACTG TACGATCAAA
```

ClaI
~~~~~~

```
7401  TACGATTACC GTTCATCGAT TCTCTTGTTT GCTCCAGACT TTCAGGTAAT
      ATGCTAATGG CAAGTAGCTA AGAGAACAAA CGAGGTCTGA AAGTCCATTA

7451  GACCTGATAG CCTTTGTAGA CCTCTCAAAA ATAGCTACCC TCTCCGGCAT
      CTGGACTATC GGAAACATCT GGAGAGTTTT TATCGATGGG AGAGGCCGTA

7501  GAATTTATCA GCTAGAACGG TTGAATATCA TATTGACGGT GATTTGACTG
      CTTAAATAGT CGATCTTGCC AACTTATAGT ATAACTGCCA CTAAACTGAC

7551  TCTCCGGCCT TTCTCACCCG TTTGAATCTT TGCCTACTCA TTACTCCGGC
      AGAGGCCGGA AAGAGTGGGC AAACTTAGAA ACGGATGAGT AATGAGGCCG

7601  ATTGCATTTA AAATATATGA GGGTTCTAAA AATTTTTATC CCTGCGTTGA
      TAACGTAAAT TTTATATACT CCCAAGATTT TTAAAAATAG GGACGCAACT

7651  AATTAAGGCT TCACCAGCAA AAGTATTACA GGGTCATAAT GTTTTTGGTA
      TTAATTCCGA AGTGGTCGTT TTCATAATGT CCCAGTATTA CAAAAACCAT

7701  CAACCGATTT AGCTTTATGC TCTGAGGCTT TATTGCTTAA TTTTGCTAAC
      GTTGGCTAAA TCGAAATACG AGACTCCGAA ATAACGAATT AAAACGATTG

7751  TCTCTGCCTT GCTTGTACGA TTTATTGGAT GTT
      AGAGACGGAA CGAACATGCT AAATAACCTA CAA
```
~~~~~~

Figure 3


Cla I (6689)
BamH I (6293)
gene II
gene X
gene V
gene VII
gene IX
gene VIII
ompA
FLAG
jun
EcoR I (2827)
Cla I (3083)
gIIIc
Hind III (3410)
f jun1B-seq
7055 bp

```
   1  AACGCTACTA CCATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC
      TTGCGATGAT GGTAATCATC TTAACTACGG TGGAAAAGTC GAGCGCGGGG

  51  AAATGAAAAT ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA
      TTTACTTTTA TATCGATTTG TCCAATAACT GGTAAACGCT TTACATAGAT

 101  ATGGTCAAAC TAAATCTACT CGTTCGCAGA ATTGGGAATC AACTGTTACA
      TACCAGTTTG ATTTAGATGA GCAAGCGTCT TAACCCTTAG TTGACAATGT

 151  TGGAATGAAA CTTCCAGACA CCGTACTTTA GTTGCATATT TAAAACATGT
      ACCTTACTTT GAAGGTCTGT GGCATGAAAT CAACGTATAA ATTTTGTACA

 201  TGAACTACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA TCCGCAAAAA
      ACTTGATGTC GTGGTCTAAG TCGTTAATTC GAGATTCGGT AGGCGTTTTT

 251  TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTGTCTAA TCCTGACCTG
      ACTGGAGAAT AGTTTTCCTC GTTAATTTCC ATGACAGATT AGGACTGGAC

 301  TTGGAATTTG CTTCCGGTCT GGTTCGCTTT GAGGCTCGAA TTGAAACGCG
      AACCTTAAAC GAAGGCCAGA CCAAGCGAAA CTCCGAGCTT AACTTTGCGC

 351  ATATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATTCGCT
      TATAAACTTC AGAAAGCCCG AAGGAGAATT AGAAAAACTA CGTTAAGCGA

 401  TTGCTTCTGA CTATAATAGA CAGGGTAAAG ACCTGATTTT TGATTTATGG
      AACGAAGACT GATATTATCT GTCCCATTTC TGGACTAAAA ACTAAATACC

 451  TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA
      AGTAAGAGCA AAAGACTTGA CAAATTTCGT AAACTCCCCC TAAGTTACTT

 501  TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
      ATAAATACTG CTAAGGCGTC ATAACCTGCG ATAGGTCAGA TTTGTAAAAT

 551  CAATTACCCC CTCTGGCAAA ACTTCCTTTG CAAAAGCCTC TCGCTATTTT
      GTTAATGGGG GAGACCGTTT TGAAGGAAAC GTTTTCGGAG AGCGATAAAA

 601  GGTTTCTATC GTCGTCTGGT TAATGAGGGT TATGATAGTG TTGCTCTTAC
      CCAAAGATAG CAGCAGACCA ATTACTCCCA ATACTATCAC AACGAGAATG

 651  CATGCCTCGT AATTCCTTTT GGCGTTATGT ATCTGCATTA GTTGAGTGTG
      GTACGGAGCA TTAAGGAAAA CCGCAATACA TAGACGTAAT CAACTCACAC

 701  GTATTCCTAA ATCTCAATTG ATGAATCTTT CCACCTGTAA TAATGTTGTT
      CATAAGGATT TAGAGTTAAC TACTTAGAAA GGTGGACATT ATTACAACAA

 751  CCGTTAGTTC GTTTTATTAA CGTAGATTTT TCCTCCCAAC GTCCTGACTG
      GGCAATCAAG CAAAATAATT GCATCTAAAA AGGAGGGTTG CAGGACTGAC

 801  GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA AAATGATTAA
      CATATTACTC GGTCAAGAAT TTTAGCGTAT TCCATTAAGT TTTACTAATT
```

```
851  AGTTGAAATT AAACCGTCTC AAGCGCAATT TACTACCCGT TCTGGTGTTT
     TCAACTTTAA TTTGGCAGAG TTCGCGTTAA ATGATGGGCA AGACCACAAA

901  CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTTG TTACGTTGAT
     GAGCAGTCCC GTTCGGAATA AGTGACTTAC TCGTCGAAAC AATGCAACTA

951  TTGGGTAATG AATATCCGGT GCTTGTCAAG ATTACTCTCG ACGAAGGTCA
     AACCCATTAC TTATAGGCCA CGAACAGTTC TAATGAGAGC TGCTTCCAGT

1001 GCCAGCGTAT GCGCCTGGTC TGTACACCGT GCATCTGTCC TCGTTCAAAG
     CGGTCGCATA CGCGGACCAG ACATGTGGCA CGTAGACAGG AGCAAGTTTC

1051 TTGGTCAGTT CGGTTCTCTT ATGATTGACC GTCTGCGCCT CGTTCCGGCT
     AACCAGTCAA GCCAAGAGAA TACTAACTGG CAGACGCGGA GCAAGGCCGA

1101 AAGTAACATG GAGCAGGTCG CGGATTTCGA CACAATTTAT CAGGCGATGA
     TTCATTGTAC CTCGTCCAGC GCCTAAAGCT GTGTTAAATA GTCCGCTACT

1151 TACAAATCTC CGTTGTACTT TGTTTCGCGC TTGGTATAAT CGCTGGGGGT
     ATGTTTAGAG GCAACATGAA ACAAAGCGCG AACCATATTA GCGACCCCCA

1201 CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTTAGGTTGG
     GTTTCTACTC ACAAAATCAC ATAAGAAAGC GGAGAAAGCA AAATCCAACC

1251 TGCCTTCGTA GTGGCATTAC GTATTTTACC CGTTTAATGG AAACTTCCTC
     ACGGAAGCAT CACCGTAATG CATAAAATGG GCAAATTACC TTTGAAGGAG

1301 ATGCGTAAGT CTTTAGTCCT CAAAGCCTCC GTAGCCGTTG CTACCCTCGT
     TACGCATTCA GAAATCAGGA GTTTCGGAGG CATCGGCAAC GATGGGAGCA

1351 TCCGATGCTG TCTTTCGCTG CTGAGGGTGA CGATCCCGCA AAAGCGGCCT
     AGGCTACGAC AGAAAGCGAC GACTCCCACT GCTAGGGCGT TTTCGCCGGA

1401 TTGACTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA TGCGTGGGCG
     AACTGAGGGA CGTTCGGAGT CGCTGGCTTA TATAGCCAAT ACGCACCCGC

1451 ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTTAAGAA
     TACCAACAAC AGTAACAGCC GCGTTGATAG CCATAGTTCG ACAAATTCTT

1501 ATTCACCTCG AAAGCAAGCT GATAAAGGAG GTTTCTCGAT CGAGACGTTN
     TAAGTGGAGC TTTCGTTCGA CTATTTCCTC CAAAGAGCTA GCTCTGCAAN

1551 NNNGAGGTTC CAACTTTCAC CATAATGAAA TAAGATCACT ACCGGGCGTA
     NNNCTCCAAG GTTGAAAGTG GTATTACTTT ATTCTAGTGA TGGCCCGCAT

1601 TTTTTTGAGT TATCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA
     AAAAAACTCA ATAGCTCTAA AAGTCCTCGA TTCCTTCGAT TTTACCTCTT

1651 AAAAATCACT GGATATACCA CCGTTGATAT ATCCCAATGG CATCGTAAAG
     TTTTTAGTGA CCTATATGGT GGCAACTATA TAGGGTTACC GTAGCATTTC
```

```
1701  AACATTTTGA GGCATTTCAG TCAGTTGCTC AATGTACCTA TAACCAGACC
      TTGTAAAACT CCGTAAAGTC AGTCAACGAG TTACATGGAT ATTGGTCTGG

1751  GTTCAGCTGG ATATTACGGC CTTTTTAAAG ACCGTAAAGA AAAATAAGCA
      CAAGTCGACC TATAATGCCG GAAAAATTTC TGGCATTTCT TTTTATTCGT

1801  CAAGTTTTAT CCGGCCTTTA TTCACATTCT TGCCCGCCTG ATGAATGCTC
      GTTCAAAATA GGCCGGAAAT AAGTGTAAGA ACGGGCGGAC TACTTACGAG

1851  ATCCGGAGTT CCGTATGGCA ATGAAAGACG GTGAGCTGGT GATATGGGAT
      TAGGCCTCAA GGCATACCGT TACTTTCTGC CACTCGACCA CTATACCCTA

1901  AGTGTTCACC CTTGTTACAC CGTTTTCCAT GAGCAAACTG AAACGTTTTC
      TCACAAGTGG GAACAATGTG GCAAAAGGTA CTCGTTTGAC TTTGCAAAAG

1951  ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT
      TAGCGAGACC TCACTTATGG TGCTGCTAAA GGCCGTCAAA GATGTGTATA

2001  ATTCGCAAGA TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA
      TAAGCGTTCT ACACCGCACA ATGCCACTTT TGGACCGGAT AAAGGGATTT

2051  GGGTTTATTG AGAATATGTT TTTCGTCTCA GCCAATCCCT GGGTGAGTTT
      CCCAAATAAC TCTTATACAA AAAGCAGAGT CGGTTAGGGA CCCACTCAAA

2101  CACCAGTTTT GATTTAAACG TAGCCAATAT GGACAACTTC TTCGCCCCCG
      GTGGTCAAAA CTAAATTTGC ATCGGTTATA CCTGTTGAAG AAGCGGGGGC

2151  TTTTCACTAT GGGCAAATAT TATACGCAAG GCGACAAGGT GCTGATGCCG
      AAAAGTGATA CCCGTTTATA ATATGCGTTC CGCTGTTCCA CGACTACGGC

2201  CTGGCGATTC AGGTTCATCA TGCCGTTTGT GATGGCTTCC ATGTCGGCAG
      GACCGCTAAG TCCAAGTAGT ACGGCAAACA CTACCGAAGG TACAGCCGTC

2251  AATGCTTAAT GAATTACAAC AGTACTGCGA TGAGTGGCAG GGCGGGGCGT
      TTACGAATTA CTTAATGTTG TCATGACGCT ACTCACCGTC CCGCCCCGCA

2301  AATTTTTTTA AGGCAGTTAT TGGTGCCCTT AAACGCCTGG TGCTAGCCTG
      TTAAAAAAAT TCCGTCAATA ACCACGGGAA TTTGCGGACC ACGATCGGAC

2351  AGGCCAGTTT GCTCAGGCTC TCCCCGTGGA GGTAATAATT GCTCGACCGA
      TCCGGTCAAA CGAGTCCGAG AGGGGCACCT CCATTATTAA CGAGCTGGCT

2401  TAAAAGCGGC TTCCTGACAG GAGGCCGTTT TGTTTTGCAG CCCACCTCAA
      ATTTTCGCCG AAGGACTGTC CTCCGGCAAA ACAAAACGTC GGGTGGAGTT

2451  CGCAATTAAT GTGAGTTAGC TCACTCATTA GGCACCCCAG GCTTTACACT
      GCGTTAATTA CACTCAATCG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA

2501  TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT
      AATACGAAGG CCGAGCATAC AACACACCTT AACACTCGCC TATTGTTAAA
```

```
2551  CACACAGGAA ACAGCTATGA CCATGATTAC GAATTTCTAG ATAACGAGGG
      GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAAAGATC TATTGCTCCC

2601  CAAAAAATGA AAAAGACAGC TATCGCGATT GCAGTGGCAC TGGCTGGTTT
      GTTTTTTACT TTTTCTGTCG ATAGCGCTAA CGTCACCGTG ACCGACCAAA

2651  CGCTACCGTA GCGCAGGCCG ACTACAAAGA TGTCGACGCC GGTGGTCGGA
      GCGATGGCAT CGCGTCCGGC TGATGTTTCT ACAGCTGCGG CCACCAGCCT

2701  TCGCCCGGCT AGAGGAAAAA GTGAAAACCT TGAAAGCGCA AAACTCCGAG
      AGCGGGCCGA TCTCCTTTTT CACTTTTGGA ACTTTCGCGT TTTGAGGCTC

2751  CTGGCGTCCA CGGCCAACAT GCTCAGGGAA CAGGTGGCAC AGCTTAAACA
      GACCGCAGGT GCCGGTTGTA CGAGTCCCTT GTCCACCGTG TCGAATTTGT

                                  EcoRI
                                  ~~~~~~~
2801  GAAAGTCATG AACCACGGTG GTGCCGAATT CAATGCTGGC GGCGGCTCTG
      CTTTCAGTAC TTGGTGCCAC CACGGCTTAA GTTACGACCG CCGCCGAGAC

2851  GTGGTGGTTC TGGTGGCGGC TCTGAGGGTG GTGGCTCTGA GGGTGGCGGT
      CACCACCAAG ACCACCGCCG AGACTCCCAC CACCGAGACT CCCACCGCCA

2901  TCTGAGGGTG GCGGCTCTGA GGGAGGCGGT TCCGGTGGTG GCTCTGGTTC
      AGACTCCCAC CGCCGAGACT CCCTCCGCCA AGGCCACCAC CGAGACCAAG

2951  CGGTGATTTT GATTATGAAA AGATGGCAAA CGCTAATAAG GGGGCTATGA
      GCCACTAAAA CTAATACTTT TCTACCGTTT GCGATTATTC CCCCGATACT

3001  CCGAAAATGC CGATGAAAAC GCGCTACAGT CTGACGCTAA AGGCAAACTT
      GGCTTTTACG GCTACTTTTG CGCGATGTCA GACTGCGATT TCCGTTTGAA

                                        ClaI
                                        ~~~~~~
3051  GATTCTGTCG CTACTGATTA CGGTGCTGCT ATCGATGGTT TCATTGGTGA
      CTAAGACAGC GATGACTAAT GCCACGACGA TAGCTACCAA AGTAACCACT

3101  CGTTTCCGGC CTTGCTAATG GTAATGGTGC TACTGGTGAT TTTGCTGGCT
      GCAAAGGCCG GAACGATTAC CATTACCACG ATGACCACTA AAACGACCGA

3151  CTAATTCCCA AATGGCTCAA GTCGGTGACG GTGATAATTC ACCTTTAATG
      GATTAAGGGT TTACCGAGTT CAGCCACTGC CACTATTAAG TGGAAATTAC

3201  AATAATTTCC GTCAATATTT ACCTTCCCTC CCTCAATCGG TTGAATGTCG
      TTATTAAAGG CAGTTATAAA TGGAAGGGAG GGAGTTAGCC AACTTACAGC

3251  CCCTTTTGTC TTTAGCGCTG GTAAACCATA TGAATTTTCT ATTGATTGTG
      GGGAAAACAG AAATCGCGAC CATTTGGTAT ACTTAAAAGA TAACTAACAC

3301  ACAAAATAAA CTTATTCCGT GGTGTCTTTG CGTTTCTTTT ATATGTTGCC
      TGTTTTATTT GAATAAGGCA CCACAGAAAC GCAAAGAAAA TATACAACGG
```

```
3351  ACCTTTATGT ATGTATTTTC TACGTTTGCT AACATACTGC GTAATAAGGA
      TGGAAATACA TACATAAAAG ATGCAAACGA TTGTATGACG CATTATTCCT

              HindIII
              ~~~~~~~
3401  GTCTTGATAA GCTTCGAGAA ATTCACCTCG AAAGCAAGCT GATAAACCGA
      CAGAACTATT CGAAGCTCTT TAAGTGGAGC TTTCGTTCGA CTATTTGGCT

3451  TACAATTAAA GGCTCCTTTT GGAGCCTTTT TTTTTGGAGA ATTAATTCAA
      ATGTTAATTT CCGAGGAAAA CCTCGGAAAA AAAAACCTCT TAATTAAGTT

3501  TCATGCCAGT TCTTTTGGGT ATTCCGTTAT TATTGCGTTT CCTCGGTTTC
      AGTACGGTCA AGAAAACCCA TAAGGCAATA ATAACGCAAA GGAGCCAAAG

3551  CTTCTGGTAA CTTTGTTCGG CTATCTGCTT ACTTTCCTTA AAAAGGGCTT
      GAAGACCATT GAAACAAGCC GATAGACGAA TGAAAGGAAT TTTTCCCGAA

3601  CGGTAAGATA GCTATTGCTA TTTCATTGTT TCTTGCTCTT ATTATTGGGC
      GCCATTCTAT CGATAACGAT AAAGTAACAA AGAACGAGAA TAATAACCCG

3651  TTAACTCAAT TCTTGTGGGT TATCTCTCTG ATATTAGCGC ACAATTACCC
      AATTGAGTTA AGAACACCCA ATAGAGAGAC TATAATCGCG TGTTAATGGG

3701  TCTGATTTTG TTCAGGGCGT TCAGTTAATT CTCCCGTCTA ATGCGCTTCC
      AGACTAAAAC AAGTCCCGCA AGTCAATTAA GAGGGCAGAT TACGCGAAGG

3751  CTGTTTTTAT GTTATTCTCT CTGTAAAGGC TGCTATTTTC ATTTTTGACG
      GACAAAAATA CAATAAGAGA GACATTTCCG ACGATAAAAG TAAAAACTGC

3801  TTAAACAAAA AATCGTTTCT TATTTGGATT GGGATAAATA AATATGGCTG
      AATTTGTTTT TTAGCAAAGA ATAAACCTAA CCCTATTTAT TTATACCGAC

3851  TTTATTTTGT AACTGGCAAA TTAGGCTCTG GAAAGACGCT CGTTAGCGTT
      AAATAAAACA TTGACCGTTT AATCCGAGAC CTTTCTGCGA GCAATCGCAA

3901  GGTAAGATTC AGGATAAAAT TGTAGCTGGG TGCAAAATAG CAACTAATCT
      CCATTCTAAG TCCTATTTTA ACATCGACCC ACGTTTTATC GTTGATTAGA

3951  TGATTTAAGG CTTCAAAACC TCCCGCAAGT CGGGAGGTTC GCTAAAACGC
      ACTAAATTCC GAAGTTTTGG AGGGCGTTCA GCCCTCCAAG CGATTTTGCG

4001  CTCGCGTTCT TAGAATACCG GATAAGCCTT CTATTTCTGA TTTGCTTGCT
      GAGCGCAAGA ATCTTATGGC CTATTCGGAA GATAAAGACT AAACGAACGA

4051  ATTGGTCGTG GTAATGATTC CTACGACGAA AATAAAAACG GTTTGCTTGT
      TAACCAGCAC CATTACTAAG GATGCTGCTT TTATTTTTGC CAAACGAACA

4101  TCTTGATGAA TGCGGTACTT GGTTTAATAC CCGTTCATGG AATGACAAGG
      AGAACTACTT ACGCCATGAA CCAAATTATG GGCAAGTACC TTACTGTTCC
```

```
4151  AAAGACAGCC GATTATTGAT TGGTTTCTTC ATGCTCGTAA ATTGGGATGG
      TTTCTGTCGG CTAATAACTA ACCAAAGAAG TACGAGCATT TAACCCTACC

4201  GATATTATTT TTCTTGTTCA GGATTTATCT ATTGTTGATA AACAGGCGCG
      CTATAATAAA AAGAACAAGT CCTAAATAGA TAACAACTAT TTGTCCGCGC

4251  TTCTGCATTA GCTGAACACG TTGTTTATTG TCGCCGTCTG GACAGAATTA
      AAGACGTAAT CGACTTGTGC AACAAATAAC AGCGGCAGAC CTGTCTTAAT

4301  CTTTACCCTT TGTCGGCACT TTATATTCTC TTGTTACTGG CTCAAAAATG
      GAAATGGGAA ACAGCCGTGA AATATAAGAG AACAATGACC GAGTTTTTAC

4351  CCTCTGCCTA AATTACATGT TGGTGTTGTT AAATATGGTG ATTCTCAATT
      GGAGACGGAT TTAATGTACA ACCACAACAA TTTATACCAC TAAGAGTTAA

4401  AAGCCCTACT GTTGAGCGTT GGCTTTATAC TGGTAAGAAT TTATATAACG
      TTCGGGATGA CAACTCGCAA CCGAAATATG ACCATTCTTA AATATATTGC

4451  CATATGACAC TAAACAGGCT TTTTCCAGTA ATTATGATTC AGGTGTTTAT
      GTATACTGTG ATTTGTCCGA AAAAGGTCAT TAATACTAAG TCCACAAATA

4501  TCATATTTAA CCCCTTATTT ATCACACGGT CGGTATTTCA AACCATTAAA
      AGTATAAATT GGGGAATAAA TAGTGTGCCA GCCATAAAGT TTGGTAATTT

4551  TTTAGGTCAG AAGATGAAAT TAACTAAAAT ATATTTGAAA AAGTTTTCTC
      AAATCCAGTC TTCTACTTTA ATTGATTTTA TATAAACTTT TTCAAAAGAG

4601  GCGTTCTTTG TCTTGCGATA GGATTTGCAT CAGCATTTAC ATATAGTTAT
      CGCAAGAAAC AGAACGCTAT CCTAAACGTA GTCGTAAATG TATATCAATA

4651  ATAACCCAAC CTAAGCCGGA GGTTAAAAAG GTAGTCTCTC AGACCTATGA
      TATTGGGTTG GATTCGGCCT CCAATTTTTC CATCAGAGAG TCTGGATACT

4701  TTTTGATAAA TTCACTATTG ACTCTTCTCA GCGTCTTAAT CTAAGCTATC
      AAAACTATTT AAGTGATAAC TGAGAAGAGT CGCAGAATTA GATTCGATAG

4751  GCTATGTTTT CAAGGATTCT AAGGGAAAAT TAATTAATAG CGACGATTTA
      CGATACAAAA GTTCCTAAGA TTCCCTTTTA ATTAATTATC GCTGCTAAAT

4801  CAGAAGCAAG GTTATTCCAT CACATATATT GATTTATGTA CTGTTTCAAT
      GTCTTCGTTC CAATAAGGTA GTGTATATAA CTAAATACAT GACAAAGTTA

4851  TAAAAAAGGT AATTCAAATG AAATTGTTAA ATGTAATTAA TTTTGTTTTC
      ATTTTTTCCA TTAAGTTTAC TTTAACAATT TACATTAATT AAAACAAAAG

4901  TTGATGTTTG TTTCATCATC TTCTTTTGCT CAAGTAATTG AAATGAATAA
      AACTACAAAC AAAGTAGTAG AAGAAAACGA GTTCATTAAC TTTACTTATT

4951  TTCGCCTCTG CGCGATTTCG TGACTTGGTA TTCAAAGCAA ACAGGTGAAT
      AAGCGGAGAC GCGCTAAAGC ACTGAACCAT AAGTTTCGTT TGTCCACTTA
```

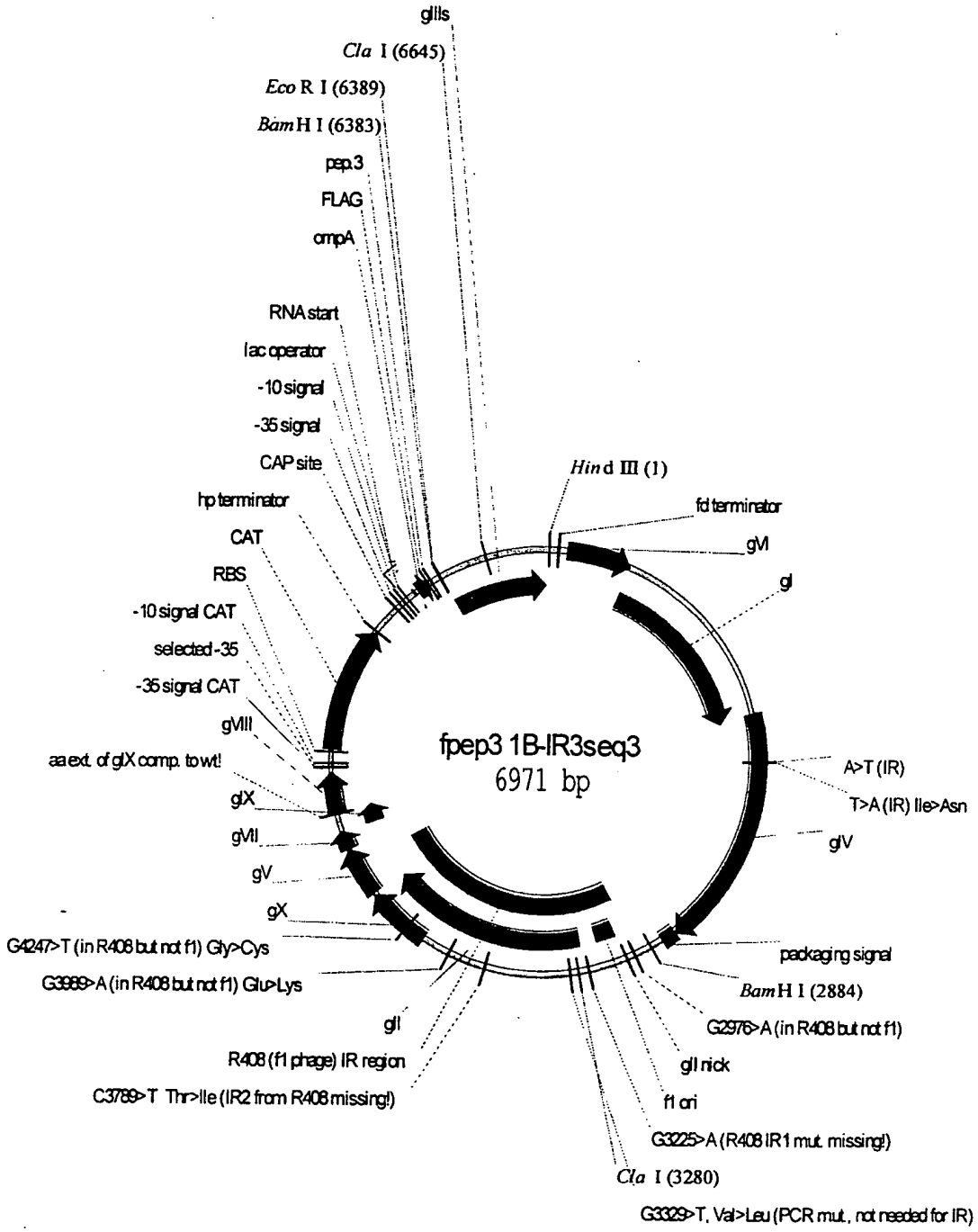
```
5001  CTGTTATTGT CTCACCTGAT GTTAAAGGTA CAGTGACTGT ATATTCCTCT
      GACAATAACA GAGTGGACTA CAATTTCCAT GTCACTGACA TATAAGGAGA

5051  GACGTTAAGC CTGAAAATTT ACGCAATTTC TTTATCTCTG TTTTACGTGC
      CTGCAATTCG GACTTTTAAA TGCGTTAAAG AAATAGAGAC AAAATGCACG

5101  TAATAATTTT GATATGGTTG GCTCAATTCC TTCCATAATT CAGAAATATA
      ATTATTAAAA CTATACCAAC CGAGTTAAGG AAGGTATTAA GTCTTTATAT

5151  ACCCAAATAG TCAGGATTAT ATTGATGAAT TGCCATCATC TGATATTCAG
      TGGGTTTATC AGTCCTAATA TAACTACTTA ACGGTAGTAG ACTATAAGTC

5201  GAATATGATG ATAATTCCGC TCCTTCTGGT GGTTTCTTTG TTCCGCAAAA
      CTTATACTAC TATTAAGGCG AGGAAGACCA CCAAAGAAAC AAGGCGTTTT

5251  TGATAATGTT ACTCAAACAT TTAAAATTAA TAACGTTCGC GCAAAGGATT
      ACTATTACAA TGAGTTTGTA AATTTTAATT ATTGCAAGCG CGTTTCCTAA

5301  TAATAAGGGT TGTAGAATTG TTTGTTAAAT CTAATACATC TAAATCCTCA
      ATTATTCCCA ACATCTTAAC AAACAATTTA GATTATGTAG ATTTAGGAGT

5351  AATGTATTAT CTGTTGATGG TTCTAACTTA TTAGTAGTTA GCGCCCCTAA
      TTACATAATA GACAACTACC AAGATTGAAT AATCATCAAT CGCGGGGATT

5401  AGATATTTTA GATAACCTTC CGCAATTTCT TTCTACTGTT GATTTGCCAA
      TCTATAAAAT CTATTGGAAG GCGTTAAAGA AAGATGACAA CTAAACGGTT

5451  CTGACCAGAT ATTGATTGAA GGATTAATTT TCGAGGTTCA GCAAGGTGAT
      GACTGGTCTA TAACTAACTT CCTAATTAAA AGCTCCAAGT CGTTCCACTA

5501  GCTTTAGATT TTTCCTTTGC TGCTGGCTCT CAGCGCGGCA CTGTTGCTGG
      CGAAATCTAA AAAGGAAACG ACGACCGAGA GTCGCGCCGT GACAACGACC

5551  TGGTGTTAAT ACTGACCGTC TAACCTCTGT TTTATCTTCT GCGGGTGGTT
      ACCACAATTA TGACTGGCAG ATTGGAGACA AAATAGAAGA CGCCCACCAA

5601  CGTTCGGTAT TTTTAACGGC GATGTTTTAG GGCTATCAGT TCGCGCATTA
      GCAAGCCATA AAAATTGCCG CTACAAAATC CCGATAGTCA AGCGCGTAAT

5651  AAGACTAATA GCCATTCAAA AATATTGTCT GTGCCTCGTA TTCTTACGCT
      TTCTGATTAT CGGTAAGTTT TTATAACAGA CACGGAGCAT AAGAATGCGA

5701  TTCAGGTCAG AAGGGTTCTA TTTCTGTTGG CCAGAATGTC CCTTTTATTA
      AAGTCCAGTC TTCCCAAGAT AAAGACAACC GGTCTTACAG GGAAAATAAT

5751  CTGGTCGTGT AACTGGTGAA TCTGCCAATG TAAATAATCC ATTTCAGACG
      GACCAGCACA TTGACCACTT AGACGGTTAC ATTTATTAGG TAAAGTCTGC

5801  GTTGAGCGTC AAAATGTTGG TATTTCTATG AGTGTTTTTC CCGTTGCAAT
      CAACTCGCAG TTTTACAACC ATAAAGATAC TCACAAAAAG GGCAACGTTA
```

```
5851  GGCTGGCGGT AATATTGTTT TAGATATAAC CAGTAAGGCC GATAGTTTGA
      CCGACCGCCA TTATAACAAA ATCTATATTG GTCATTCCGG CTATCAAACT

5901  GTTCTTCTAC TCAGGCAAGT GATGTTATTA CTAATCAAAG AAGTATTGCG
      CAAGAAGATG AGTCCGTTCA CTACAATAAT GATTAGTTTC TTCATAACGC

5951  ACAACGGTTA ATTTGCGTGA TGGTCAGACT CTTTTGCTCG GTGGCCTCAC
      TGTTGCCAAT TAAACGCACT ACCAGTCTGA GAAAACGAGC CACCGGAGTG

6001  TGATTACAAA AACACTTCTC AAGATTCTGG TGTGCCGTTC CTGTCTAAAA
      ACTAATGTTT TTGTGAAGAG TTCTAAGACC ACACGGCAAG GACAGATTTT

6051  TCCCTTTAAT CGGCCTCCTG TTTAGCTCCC GTTCTGATTC TAACGAGGAA
      AGGGAAATTA GCCGGAGGAC AAATCGAGGG CAAGACTAAG ATTGCTCCTT

6101  AGCACGTTGT ACGTGCTCGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG
      TCGTGCAACA TGCACGAGCA GTTTCGTTGG TATCATGCGC GGGACATCGC

6151  GCGCATTAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA
      CGCGTAATTC GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT

6201  CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT
      GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA

                                                    BamHI
                                                    ------
6251  CGCCACGTTC TCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGATCCCTT
      GCGGTGCAAG AGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCTAGGGAA

6301  TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCTCCA AAAACTTGAT
      ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGAGGT TTTTGAACTA

6351  TTGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG
      AACCCACTAC CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC

6401  CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA
      GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT

6451  CTGGAACAAC ACTCACAACT AACTCGGCCT ATTCTTTTGA TTTATAAGGA
      GACCTTGTTG TGAGTGTTGA TTGAGCCGGA TAAGAAAACT AAATATTCCT

6501  TTTTTGTCAT TTTCTGCTTA CTGGTTAAAA AATAAGCTGA TTTAACAAAT
      AAAAACAGTA AAAGACGAAT GACCAATTTT TTATTCGACT AAATTGTTTA

6551  ATTTAACGCG AAATTTAACA AAACATTAAC GTTTACAATT TAAATATTTG
      TAAATTGCGC TTTAAATTGT TTTGTAATTG CAAATGTTAA ATTTATAAAC

6601  CTTATACAAT CATCCTGTTT TTGGGGCTTT TCTGATTATC AACCGGGGTA
      GAATATGTTA GTAGGACAAA AACCCCGAAA AGACTAATAG TTGGCCCCAT
```

ClaI
~~~~~~~

```
6651  CATATGATTG ACATGCTAGT TTTACGATTA CCGTTCATCG ATTCTCTTGT
      GTATACTAAC TGTACGATCA AAATGCTAAT GGCAAGTAGC TAAGAGAACA

6701  TTGCTCCAGA CTTTCAGGTA ATGACCTGAT AGCCTTTGTA GACCTCTCAA
      AACGAGGTCT GAAAGTCCAT TACTGGACTA TCGGAAACAT CTGGAGAGTT

6751  AAATAGCTAC CCTCTCCGGC ATGAATTTAT CAGCTAGAAC GGTTGAATAT
      TTTATCGATG GGAGAGGCCG TACTTAAATA GTCGATCTTG CCAACTTATA

6801  CATATTGACG GTGATTTGAC TGTCTCCGGC CTTTCTCACC CGTTTGAATC
      GTATAACTGC CACTAAACTG ACAGAGGCCG GAAAGAGTGG GCAAACTTAG

6851  TTTGCCTACT CATTACTCCG GCATTGCATT TAAAATATAT GAGGGTTCTA
      AAACGGATGA GTAATGAGGC CGTAACGTAA ATTTTATATA CTCCCAAGAT

6901  AAAATTTTTA TCCCTGCGTT GAAATTAAGG CTTCACCAGC AAAAGTATTA
      TTTTAAAAAT AGGGACGCAA CTTTAATTCC GAAGTGGTCG TTTTCATAAT

6951  CAGGGTCATA ATGTTTTTGG TACAACCGAT TTAGCTTTAT GCTCTGAGGC
      GTCCCAGTAT TACAAAAACC ATGTTGGCTA AATCGAAATA CGAGACTCCG

7001  TTTATTGCTT AATTTTGCTA ACTCTCTGCC TTGCTTGTAC GATTTATTGG
      AAATAACGAA TTAAAACGAT TGAGAGACGG AACGAACATG CTAAATAACC

7051  ATGTT
      TACAA
```
~~~~~~~

Figure 4


gIIs
Cla I (6645)
Eco R I (6389)
BamH I (6383)
pep.3
FLAG
ompA
RNA start
lac operator
-10 signal
-35 signal
CAP site
hp terminator
CAT
RBS
-10 signal CAT
selected -35
-35 signal CAT
gVIII
aa ext. of gIX comp. to wt
gIX
gVII
gV
gX
G4247>T (in R408 but not f1) Gly>Cys
G3989>A (in R408 but not f1) Glu>Lys
gII
R408 (f1 phage) IR region
C3789>T Thr>Ile (IR2 from R408 missing!)
Hind III (1)
fd terminator
gVI
gI
fpep3 1B-IR3seq3
6971 bp
A>T (IR)
T>A (IR) Ile>Asn
gIV
packaging signal
BamH I (2884)
G2976>A (in R408 but not f1)
gII nick
f1 ori
G3225>A (R408 IR1 mut. missing!)
Cla I (3280)
G3329>T, Val>Leu (PCR mut., not needed for IR)

HindIII
~~~~~

```
   1  AGCTTCGAGA AATTCACCTC GAAAGCAAGC TGATAAACCG ATACAATTAA
      TCGAAGCTCT TTAAGTGGAG CTTTCGTTCG ACTATTTGGC TATGTTAATT

  51  AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG AATTAATTCA ATCATGCCAG
      TCCGAGGAAA ACCTCGGAAA AAAAAACCTC TTAATTAAGT TAGTACGGTC

 101  TTCTTTTGGG TATTCCGTTA TTATTGCGTT TCCTCGGTTT CCTTCTGGTA
      AAGAAAACCC ATAAGGCAAT AATAACGCAA AGGAGCCAAA GGAAGACCAT

 151  ACTTTGTTCG GCTATCTGCT TACTTTCCTT AAAAAGGGCT TCGGTAAGAT
      TGAAACAAGC CGATAGACGA ATGAAAGGAA TTTTTCCCGA AGCCATTCTA

 201  AGCTATTGCT ATTTCATTGT TTCTTGCTCT TATTATTGGG CTTAACTCAA
      TCGATAACGA TAAAGTAACA AAGAACGAGA ATAATAACCC GAATTGAGTT

 251  TTCTTGTGGG TTATCTCTCT GATATTAGCG CACAATTACC CTCTGATTTT
      AAGAACACCC AATAGAGAGA CTATAATCGC GTGTTAATGG GAGACTAAAA

 301  GTTCAGGGCG TTCAGTTAAT TCTCCCGTCT AATGCGCTTC CCTGTTTTTA
      CAAGTCCCGC AAGTCAATTA AGAGGGCAGA TTACGCGAAG GGACAAAAAT

 351  TGTTATTCTC TCTGTAAAGG CTGCTATTTT CATTTTTGAC GTTAAACAAA
      ACAATAAGAG AGACATTTCC GACGATAAAA GTAAAAACTG CAATTTGTTT

 401  AAATCGTTTC TTATTTGGAT TGGGATAAAT AAATATGGCT GTTTATTTTG
      TTTAGCAAAG AATAAACCTA ACCCTATTTA TTTATACCGA CAAATAAAAC

 451  TAACTGGCAA ATTAGGCTCT GGAAAGACGC TCGTTAGCGT TGGTAAGATT
      ATTGACCGTT TAATCCGAGA CCTTTCTGCG AGCAATCGCA ACCATTCTAA

 501  CAGGATAAAA TTGTAGCTGG GTGCAAAATA GCAACTAATC TTGATTTAAG
      GTCCTATTTT AACATCGACC CACGTTTTAT CGTTGATTAG AACTAAATTC

 551  GCTTCAAAAC CTCCCGCAAG TCGGGAGGTT CGCTAAAACG CCTCGCGTTC
      CGAAGTTTTG GAGGGCGTTC AGCCCTCCAA GCGATTTTGC GGAGCGCAAG

 601  TTAGAATACC GGATAAGCCT TCTATTTCTG ATTTGCTTGC TATTGGTCGT
      AATCTTATGG CCTATTCGGA AGATAAAGAC TAAACGAACG ATAACCAGCA

 651  GGTAATGATT CCTACGACGA AAATAAAAAC GGTTTGCTTG TTCTTGATGA
      CCATTACTAA GGATGCTGCT TTTATTTTTG CCAAACGAAC AAGAACTACT

 701  ATGCGGTACT TGGTTTAATA CCCGTTCATG GAATGACAAG GAAAGACAGC
      TACGCCATGA ACCAAATTAT GGGCAAGTAC CTTACTGTTC CTTTCTGTCG

 751  CGATTATTGA TTGGTTTCTT CATGCTCGTA AATTGGGATG GGATATTATT
      GCTAATAACT AACCAAAGAA GTACGAGCAT TTAACCCTAC CCTATAATAA
```
~~~~~

```
801  TTTCTTGTTC AGGATTTATC TATTGTTGAT AAACAGGCGC GTTCTGCATT
     AAAGAACAAG TCCTAAATAG ATAACAACTA TTTGTCCGCG CAAGACGTAA

851  AGCTGAACAC GTTGTTTATT GTCGCCGTCT GGACAGAATT ACTTTACCCT
     TCGACTTGTG CAACAAATAA CAGCGGCAGA CCTGTCTTAA TGAAATGGGA

901  TTGTCGGCAC TTTATATTCT CTTGTTACTG GCTCAAAAAT GCCTCTGCCT
     AACAGCCGTG AAATATAAGA GAACAATGAC CGAGTTTTTA CGGAGACGGA

951  AAATTACATG TTGGTGTTGT TAAATATGGT GATTCTCAAT TAAGCCCTAC
     TTTAATGTAC AACCACAACA ATTTATACCA CTAAGAGTTA ATTCGGGATG

1001 TGTTGAGCGT TGGCTTTATA CTGGTAAGAA TTTATATAAC GCATATGACA
     ACAACTCGCA ACCGAAATAT GACCATTCTT AAATATATTG CGTATACTGT

1051 CTAAACAGGC TTTTTCCAGT AATTATGATT CAGGTGTTTA TTCATATTTA
     GATTTGTCCG AAAAAGGTCA TTAATACTAA GTCCACAAAT AAGTATAAAT

1101 ACCCCTTATT TATCACACGG TCGGTATTTC AAACCATTAA ATTTAGGTCA
     TGGGGAATAA ATAGTGTGCC AGCCATAAAG TTTGGTAATT TAAATCCAGT

1151 GAAGATGAAA TTAACTAAAA TATATTTGAA AAAGTTTTCT CGCGTTCTTT
     CTTCTACTTT AATTGATTTT ATATAAACTT TTTCAAAAGA GCGCAAGAAA

1201 GTCTTGCGAT AGGATTTGCA TCAGCATTTA CATATAGTTA TATAACCCAA
     CAGAACGCTA TCCTAAACGT AGTCGTAAAT GTATATCAAT ATATTGGGTT

1251 CCTAAGCCGG AGGTTAAAAA GGTAGTCTCT CAGACCTATG ATTTTGATAA
     GGATTCGGCC TCCAATTTTT CCATCAGAGA GTCTGGATAC TAAAACTATT

1301 ATTCACTATT GACTCTTCTC AGCGTCTTAA TCTAAGCTAT CGCTATGTTT
     TAAGTGATAA CTGAGAAGAG TCGCAGAATT AGATTCGATA GCGATACAAA

1351 TCAAGGATTC TAAGGGAAAA TTAATTAATA GCGACGATTT ACAGAAGCAA
     AGTTCCTAAG ATTCCCTTTT AATTAATTAT CGCTGCTAAA TGTCTTCGTT

1401 GGTTATTCCA TCACATATAT TGATTTATGT ACTGTTTCAA TTAAAAAAGG
     CCAATAAGGT AGTGTATATA ACTAAATACA TGACAAAGTT AATTTTTTCC

1451 TAATTCAAAT GAAATTGTTA AATGTAATTA ATTTTGTTTT CTTGATGTTT
     ATTAAGTTTA CTTTAACAAT TTACATTAAT TAAAACAAAA GAACTACAAA

1501 GTTTCATCAT CTTCTTTTGC TCAAGTAATT GAAATGAATA ATTCGCCTCT
     CAAAGTAGTA GAAGAAAACG AGTTCATTAA CTTTACTTAT TAAGCGGAGA

1551 GCGCGATTTC GTGACTTGGT ATTCAAAGCA AACAGGTGAA TCTGTTATTG
     CGCGCTAAAG CACTGAACCA TAAGTTTCGT TTGTCCACTT AGACAATAAC

1601 TCTCACCTGA TGTTAAAGGT ACAGTGACTG TATATTCCTC TGACGTTAAG
     AGAGTGGACT ACAATTTCCA TGTCACTGAC ATATAAGGAG ACTGCAATTC
```

```
1651  CCTGAAAATT TACGCAATTT CTTTATCTCT GTTTTACGTG CTAATAATTT
      GGACTTTTAA ATGCGTTAAA GAAATAGAGA CAAAATGCAC GATTATTAAA

1701  TGATATGGTT GGCTCTAATC CTTCCATAAT TCAGAAATAT AACCCAAATA
      ACTATACCAA CCGAGATTAG GAAGGTATTA AGTCTTTATA TTGGGTTTAT

1751  GTCAGGATTA TATTGATGAA TTGCCATCAT CTGATATTCA GGAATATGAT
      CAGTCCTAAT ATAACTACTT AACGGTAGTA GACTATAAGT CCTTATACTA

1801  GATAATTCCG CTCCTTCTGG TGGTTTCTTT GTTCCGCAAA ATGATAATGT
      CTATTAAGGC GAGGAAGACC ACCAAAGAAA CAAGGCGTTT TACTATTACA

1851  TACTCAAACA TTTAAAATTA ATAACGTTCG CGCAAAGGAT TTAATAAGGG
      ATGAGTTTGT AAATTTTAAT TATTGCAAGC GCGTTTCCTA AATTATTCCC

1901  TTGTAGAATT GTTTGTTAAA TCTAATACAT CTAAATCCTC AAATGTATTA
      AACATCTTAA CAAACAATTT AGATTATGTA GATTTAGGAG TTTACATAAT

1951  TCTGTTGATG GTTCTAACTT ATTAGTAGTT AGCGCCCCTA AAGATATTTT
      AGACAACTAC CAAGATTGAA TAATCATCAA TCGCGGGGAT TTCTATAAAA

2001  AGATAACCTT CCGCAATTTC TTTCTACTGT TGATTTGCCA ACTGACCAGA
      TCTATTGGAA GGCGTTAAAG AAAGATGACA ACTAAACGGT TGACTGGTCT

2051  TATTGATTGA AGGATTAATT TTCGAGGTTC AGCAAGGTGA TGCTTTAGAT
      ATAACTAACT TCCTAATTAA AAGCTCCAAG TCGTTCCACT ACGAAATCTA

2101  TTTTCCTTTG CTGCTGGCTC TCAGCGCGGC ACTGTTGCTG GTGGTGTTAA
      AAAAGGAAAC GACGACCGAG AGTCGCGCCG TGACAACGAC CACCACAATT

2151  TACTGACCGT CTAACCTCTG TTTTATCTTC TGCGGGTGGT TCGTTCGGTA
      ATGACTGGCA GATTGGAGAC AAAATAGAAG ACGCCCACCA AGCAAGCCAT

2201  TTTTTAACGG CGATGTTTTA GGGCTATCAG TTCGCGCATT AAAGACTAAT
      AAAAATTGCC GCTACAAAAT CCCGATAGTC AAGCGCGTAA TTTCTGATTA

2251  AGCCATTCAA AAATATTGTC TGTGCCTCGT ATTCTTACGC TTTCAGGTCA
      TCGGTAAGTT TTTATAACAG ACACGGAGCA TAAGAATGCG AAAGTCCAGT

2301  GAAGGGTTCT ATTTCTGTTG GCCAGAATGT CCCTTTTATT ACTGGTCGTG
      CTTCCCAAGA TAAAGACAAC CGGTCTTACA GGGAAAATAA TGACCAGCAC

2351  TAACTGGTGA ATCTGCCAAT GTAAATAATC CATTTCAGAC AATTGAGCGT
      ATTGACCACT TAGACGGTTA CATTTATTAG GTAAAGTCTG TTAACTCGCA

2401  CAAAATGTTG GTATTTCTAT GAGTGTTTTT CCCGTTGCAA TGGCTGGCGG
      GTTTTACAAC CATAAAGATA CTCACAAAAA GGGCAACGTT ACCGACCGCC

2451  TAATATTGTT TTAGATATAA CCAGTAAGGC CGATAGTTTG AGTTCTTCTA
      ATTATAACAA AATCTATATT GGTCATTCCG GCTATCAAAC TCAAGAAGAT
```

```
2501  CTCAGGCAAG TGATGTTATT ACTAATCAAA GAAGTATTGC GACAACGGTT
      GAGTCCGTTC ACTACAATAA TGATTAGTTT CTTCATAACG CTGTTGCCAA

2551  AATTTGCGTG ATGGTCAGAC TCTTTTGCTC GGTGGCCTCA CTGATTACAA
      TTAAACGCAC TACCAGTCTG AGAAAACGAG CCACCGGAGT GACTAATGTT

2601  AAACACTTCT CAAGATTCTG GTGTGCCGTT CCTGTCTAAA ATCCCTTTAA
      TTTGTGAAGA GTTCTAAGAC CACACGGCAA GGACAGATTT TAGGGAAATT

2651  TCGGCCTCCT GTTTAGCTCC CGTTCTGATT CTAACGAGGA AAGCACGTTG
      AGCCGGAGGA CAAATCGAGG GCAAGACTAA GATTGCTCCT TTCGTGCAAC

2701  TACGTGCTCG TCAAAGCAAC CATAGTACGC GCCCTGTAGC GGCGCATTAA
      ATGCACGAGC AGTTTCGTTG GTATCATGCG CGGGACATCG CCGCGTAATT

2751  GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC
      CGCGCCGCCC ACACCACCAA TGCGCGTCGC ACTGGCGATG TGAACGGTCG

2801  GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CCTTCCTTTC TCGCCACGTT
      CGGGATCGCG GGCGAGGAAA GCGAAAGAAG GGAAGGAAAG AGCGGTGCAA

                                          BamHI
                                          ------
2851  CTCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGATCCCT TTAGGGTTCC
      GAGGCCGAAA GGGGCAGTTC GAGATTTAGC CCCCTAGGGA AATCCCAAGG

2901  GATTTAGTGC TTTACGGCAC CTCGACCTCC AAAAACTTGA TTTGGGTGAT
      CTAAATCACG AAATGCCGTG GAGCTGGAGG TTTTTGAACT AAACCCACTA

2951  GGTTCACGTA GTGGGCCATC GCCCTAATAG ACGGTTTTTC GCCCTTTGAC
      CCAAGTGCAT CACCCGGTAG CGGGATTATC TGCCAAAAAG CGGGAAACTG

3001  GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA ACTGGAACAA
      CAACCTCAGG TGCAAGAAAT TATCACCTGA GAACAAGGTT TGACCTTGTT

3051  CACTCAACCC TATCTCGGTC TATTCTTTTG ATTTATAAGG GATTTTGCCG
      GTGAGTTGGG ATAGAGCCAG ATAAGAAAAC TAAATATTCC CTAAAACGGC

3101  ATTTCGGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC
      TAAAGCCGGA TAACCAATTT TTTACTCGAC TAAATTGTTT TTAAATTGCG

3151  GAATTTTAAC AAAATATTAA CGTTTACAAT TTAAATATTT GCTTATACAA
      CTTAAAATTG TTTTATAATT GCAAATGTTA AATTTATAAA CGAATATGTT

3201  TCTTCCTGTT TTTGGGGCTT TTCTGATTAT CAACCGGGGT ACATATGATT
      AGAAGGACAA AAACCCCGAA AAGACTAATA GTTGGCCCCA TGTATACTAA

                                       ClaI
                                       -------
3251  GACATGCTAG TTTTACGATT ACCGTTCATC GATTCTCTTG TTTGCTCCAG
      CTGTACGATC AAAATGCTAA TGGCAAGTAG CTAAGAGAAC AAACGAGGTC
```

```
3301  ACTCTCAGGC AATGACCTGA TAGCCTTTTT AGACCTCTCA AAAATAGCTA
      TGAGAGTCCG TTACTGGACT ATCGGAAAAA TCTGGAGAGT TTTTATCGAT

3351  CCCTCTCCGG CATGAATTTA TCAGCTAGAA CGGTTGAATA TCATATTGAT
      GGGAGAGGCC GTACTTAAAT AGTCGATCTT GCCAACTTAT AGTATAACTA

3401  GGTGATTTGA CTGTCTCCGG CCTTTCTCAC CCGTTTGAAT CTTTACCTAC
      CCACTAAACT GACAGAGGCC GGAAAGAGTG GGCAAACTTA GAAATGGATG

3451  ACATTACTCA GGCATTGCAT TTAAAATATA TGAGGGTTCT AAAAATTTTT
      TGTAATGAGT CCGTAACGTA AATTTTATAT ACTCCCAAGA TTTTTAAAAA

3501  ATCCTTGCGT TGAAATAAAG GCTTCTCCCG CAAAAGTATT ACAGGGTCAT
      TAGGAACGCA ACTTTATTTC CGAAGAGGGC GTTTTCATAA TGTCCCAGTA

3551  AATGTTTTTG GTACAACCGA TTTAGCTTTA TGCTCTGAGG CTTTATTGCT
      TTACAAAAAC CATGTTGGCT AAATCGAAAT ACGAGACTCC GAAATAACGA

3601  TAATTTTGCT AATTCTTTGC CTTGCCTGTA TGATTTATTG GATGTTAACG
      ATTAAAACGA TTAAGAAACG GAACGGACAT ACTAAATAAC CTACAATTGC

3651  CTACTACTAT TAGTAGAATT GATGCCACCT TTTCAGCTCG CGCCCCAAAT
      GATGATGATA ATCATCTTAA CTACGGTGGA AAAGTCGAGC GCGGGGTTTA

3701  GAAAATATAG CTAAACAGGT TATTGACCAT TTGCGAAATG TATCTAATGG
      CTTTTATATC GATTTGTCCA ATAACTGGTA AACGCTTTAC ATAGATTACC

3751  TCAAACTAAA TCTACTCGTT CGCAGAATTG GGAATCAACT GTTACATGGA
      AGTTTGATTT AGATGAGCAA GCGTCTTAAC CCTTAGTTGA CAATGTACCT

3801  ATGAAACTTC CAGACACCGT ACTTTAGTTG CATATTTAAA ACATGTTGAG
      TACTTTGAAG GTCTGTGGCA TGAAATCAAC GTATAAATTT TGTACAACTC

3851  CTACAGCACC AGATCCAGCA ATTAAGCTCT AAGCCATCCG CAAAAATGAC
      GATGTCGTGG TCTAGGTCGT TAATTCGAGA TTCGGTAGGC GTTTTTACTG

3901  CTCTTATCAA AAGGAGCAAT TAAAGGTACT CTCTAATCCT GACCTGTTGG
      GAGAATAGTT TTCCTCGTTA ATTTCCATGA GAGATTAGGA CTGGACAACC

3951  AGTTTGCTTC CGGTCTGGTT CGCTTTGAAG CTCGAATTAA AACGCGATAT
      TCAAACGAAG GCCAGACCAA GCGAAACTTC GAGCTTAATT TTGCGCTATA

4001  TTGAAGTCTT TCGGGCTTCC TCTTAATCTT TTTGATGCAA TCCGCTTTGC
      AACTTCAGAA AGCCCGAAGG AGAATTAGAA AAACTACGTT AGGCGAAACG

4051  TTCTGACTAT AATAGTCAGG GTAAAGACCT GATTTTTGAT TTATGGTCAT
      AAGACTGATA TTATCAGTCC CATTTCTGGA CTAAAAACTA AATACCAGTA

4101  TCTCGTTTTC TGAACTGTTT AAAGCATTTG AGGGGGATTC AATGAATATT
      AGAGCAAAAG ACTTGACAAA TTTCGTAAAC TCCCCCTAAG TTACTTATAA
```

```
4151  TATGACGATT CCGCAGTATT GGACGCTATC CAGTCTAAAC ATTTTACTAT
      ATACTGCTAA GGCGTCATAA CCTGCGATAG GTCAGATTTG TAAAATGATA

4201  TACCCCCTCT GGCAAAACTT CTTTTGCAAA AGCCTCTCGC TATTTTTGTT
      ATGGGGGAGA CCGTTTTGAA GAAAACGTTT TCGGAGAGCG ATAAAAACAA

4251  TTTATCGTCG TCTGGTAAAC GAGGGTTATG ATAGTGTTGC TCTTACTATG
      AAATAGCAGC AGACCATTTG CTCCCAATAC TATCACAACG AGAATGATAC

4301  CCTCGTAATT CCTTTTGGCG TTATGTATCT GCATTAGTTG AATGTGGTAT
      GGAGCATTAA GGAAAACCGC AATACATAGA CGTAATCAAC TTACACCATA

4351  TCCTAAATCT CAACTGATGA ATCTTTCTAC CTGTAATAAT GTTGTTCCGT
      AGGATTTAGA GTTGACTACT TAGAAAGATG GACATTATTA CAACAAGGCA

4401  TAGTTCGTTT TATTAACGTA GATTTTTCTT CCCAACGTCC TGACTGGTAT
      ATCAAGCAAA ATAATTGCAT CTAAAAAGAA GGGTTGCAGG ACTGACCATA

4451  AATGAGCCAG TTCTTAAAAT CGCATAAGGT AATTCACAAT GATTAAAGTT
      TTACTCGGTC AAGAATTTTA GCGTATTCCA TTAAGTGTTA CTAATTTCAA

4501  GAAATTAAAC CATCTCAAGC GCAATTCACT ACCCGTTCTG GTGTTTCTCG
      CTTTAATTTG GTAGAGTTCG CGTTAAGTGA TGGGCAAGAC CACAAAGAGC

4551  TCAGGGCAAG CCTTATTCAC TGAATGAGCA GCTTTGTTAC GTTGATTTGG
      AGTCCCGTTC GGAATAAGTG ACTTACTCGT CGAAACAATG CAACTAAACC

4601  GTAATGAATA TCCGGTGCTT GTCAAGATTA CTCTTGATGA AGGTCAGCCA
      CATTACTTAT AGGCCACGAA CAGTTCTAAT GAGAACTACT TCCAGTCGGT

4651  GCCTATGCGC CTGGTCTGTA CACCGTGCAT CTGTCCTCGT TCAAAGTTGG
      CGGATACGCG GACCAGACAT GTGGCACGTA GACAGGAGCA AGTTTCAACC

4701  TCAGTTCGGT TCTCTTATGA TTGACCGTCT GCGCCTCGTT CCGGCTAAGT
      AGTCAAGCCA AGAGAATACT AACTGGCAGA CGCGGAGCAA GGCCGATTCA

4751  AACATGGAGC AGGTCGCGGA TTTCGACACA ATTTATCAGG CGATGATACA
      TTGTACCTCG TCCAGCGCCT AAAGCTGTGT TAAATAGTCC GCTACTATGT

4801  AATCTCCGTT GTACTTTGTT TCGCGCTTGG TATAATCGCT GGGGGTCAAA
      TTAGAGGCAA CATGAAACAA AGCGCGAACC ATATTAGCGA CCCCCAGTTT

4851  GATGAGTGTT TTAGTGTATT CTTTCGCCTC TTTCGTTTTA GGTTGGTGCC
      CTACTCACAA AATCACATAA GAAAGCGGAG AAAGCAAAAT CCAACCACGG

4901  TTCGTAGTGG CATTACGTAT TTTACCCGTT TAATGGAAAC TTCCTCATGC
      AAGCATCACC GTAATGCATA AAATGGGCAA ATTACCTTTG AAGGAGTACG

4951  GTAAGTCTTT AGTCCTCAAA GCCTCCGTAG CCGTTGCTAC CCTCGTTCCG
      CATTCAGAAA TCAGGAGTTT CGGAGGCATC GGCAACGATG GGAGCAAGGC
```

```
5001  ATGCTGTCTT TCGCTGCTGA GGGTGACGAT CCCGCAAAAG CGGCCTTTGA
      TACGACAGAA AGCGACGACT CCCACTGCTA GGGCGTTTTC GCCGGAAACT

5051  CTCCCTGCAA GCCTCAGCGA CCGAATATAT CGGTTATGCG TGGGCGATGG
      GAGGGACGTT CGGAGTCGCT GGCTTATATA GCCAATACGC ACCCGCTACC

5101  TTGTTGTCAT TGTCGGCGCA ACTATCGGTA TCAAGCTGTT TAAGAAATTC
      AACAACAGTA ACAGCCGCGT TGATAGCCAT AGTTCGACAA ATTCTTTAAG

5151  ACCTCGAAAG CAAGCTGATA AAGGAGGTTT CTCGATCGAG ACGTTGGGTG
      TGGAGCTTTC GTTCGACTAT TTCCTCCAAA GAGCTAGCTC TGCAACCCAC

5201  AGGTTCCAAC TTTCACCATA ATGAAATAAG ATCACTACCG GGCGTATTTT
      TCCAAGGTTG AAAGTGGTAT TACTTTATTC TAGTGATGGC CCGCATAAAA

5251  TTGAGTTATC GAGATTTTCA GGAGCTAAGG AAGCTAAAAT GGAGAAAAAA
      AACTCAATAG CTCTAAAAGT CCTCGATTCC TTCGATTTTA CCTCTTTTTT

5301  ATCACTGGAT ATACCACCGT TGATATATCC CAATGGCATC GTAAAGAACA
      TAGTGACCTA TATGGTGGCA ACTATATAGG GTTACCGTAG CATTTCTTGT

5351  TTTTGAGGCA TTTCAGTCAG TTGCTCAATG TACCTATAAC CAGACCGTTC
      AAAACTCCGT AAAGTCAGTC AACGAGTTAC ATGGATATTG GTCTGGCAAG

5401  AGCTGGATAT TACGGCCTTT TTAAAGACCG TAAAGAAAAA TAAGCACAAG
      TCGACCTATA ATGCCGGAAA AATTTCTGGC ATTTCTTTTT ATTCGTGTTC

5451  TTTTATCCGG CCTTTATTCA CATTCTTGCC CGCCTGATGA ATGCTCATCC
      AAAATAGGCC GGAAATAAGT GTAAGAACGG GCGGACTACT TACGAGTAGG

5501  GGAGTTCCGT ATGGCAATGA AAGACGGTGA GCTGGTGATA TGGGATAGTG
      CCTCAAGGCA TACCGTTACT TTCTGCCACT CGACCACTAT ACCCTATCAC

5551  TTCACCCTTG TTACACCGTT TTCCATGAGC AAACTGAAAC GTTTTCATCG
      AAGTGGGAAC AATGTGGCAA AAGGTACTCG TTTGACTTTG CAAAAGTAGC

5601  CTCTGGAGTG AATACCACGA CGATTTCCGG CAGTTTCTAC ACATATATTC
      GAGACCTCAC TTATGGTGCT GCTAAAGGCC GTCAAAGATG TGTATATAAG

5651  GCAAGATGTG GCGTGTTACG GTGAAAACCT GGCCTATTTC CCTAAAGGGT
      CGTTCTACAC CGCACAATGC CACTTTTGGA CCGGATAAAG GGATTTCCCA

5701  TTATTGAGAA TATGTTTTTC GTCTCAGCCA ATCCCTGGGT GAGTTTCACC
      AATAACTCTT ATACAAAAAG CAGAGTCGGT TAGGGACCCA CTCAAAGTGG

5751  AGTTTTGATT TAAACGTAGC CAATATGGAC AACTTCTTCG CCCCCGTTTT
      TCAAAACTAA ATTTGCATCG GTTATACCTG TTGAAGAAGC GGGGGCAAAA

5801  CACTATGGGC AAATATTATA CGCAAGGCGA CAAGGTGCTG ATGCCGCTGG
      GTGATACCCG TTTATAATAT GCGTTCCGCT GTTCCACGAC TACGGCGACC
```

```
5851  CGATTCAGGT TCATCATGCC GTTTGTGATG GCTTCCATGT CGGCAGAATG
      GCTAAGTCCA AGTAGTACGG CAAACACTAC CGAAGGTACA GCCGTCTTAC

5901  CTTAATGAAT TACAACAGTA CTGCGATGAG TGGCAGGGCG GGGCGTAATT
      GAATTACTTA ATGTTGTCAT GACGCTACTC ACCGTCCCGC CCCGCATTAA

5951  TTTTTAAGGC AGTTATTGGT GCCCTTAAAC GCCTGGTGCT AGCCTGAGGC
      AAAAATTCCG TCAATAACCA CGGGAATTTG CGGACCACGA TCGGACTCCG

6001  CAGTTTGCTC AGGCTCTCCC CGTGGAGGTA ATAATTGCTC GACCGATAAA
      GTCAAACGAG TCCGAGAGGG GCACCTCCAT TATTAACGAG CTGGCTATTT

6051  AGCGGCTTCC TGACAGGAGG CCGTTTTGTT TTGCAGCCCA CCTCAACGCA
      TCGCCGAAGG ACTGTCCTCC GGCAAAACAA AACGTCGGGT GGAGTTGCGT

6101  ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT
      TAATTACACT CAATCGAGTG AGTAATCCGT GGGGTCCGAA ATGTGAAATA

6151  GCTTCCGGCT CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA
      CGAAGGCCGA GCATACAACA CACCTTAACA CTCGCCTATT GTTAAAGTGT

6201  CAGGAAACAG CTATGACCAT GATTACGAAT TTCTAGATAA CGAGGGCAAA
      GTCCTTTGTC GATACTGGTA CTAATGCTTA AAGATCTATT GCTCCCGTTT

6251  AAATGAAAAA GACAGCTATC GCGATTGCAG TGGCACTGGC TGGTTTCGCT
      TTTACTTTTT CTGTCGATAG CGCTAACGTC ACCGTGACCG ACCAAAGCGA

6301  ACCGTAGCGC AGGCCGACTA CAAAGATGTC GACTGTATTG TTTATCATGC
      TGGCATCGCG TCCGGCTGAT GTTTCTACAG CTGACATAAC AAATAGTACG

                                          BamHI EcoRI
                                          ~~~~~~~~~~~~
6351  TCATTATCTT GTTGCTAAGT GTGGTGGTGG AGGATCCGAA TTCAATGCTG
      AGTAATAGAA CAACGATTCA CACCACCACC TCCTAGGCTT AAGTTACGAC

6401  GCGGCGGCTC TGGTGGTGGT TCTGGTGGCG GCTCTGAGGG TGGTGGCTCT
      CGCCGCCGAG ACCACCACCA AGACCACCGC CGAGACTCCC ACCACCGAGA

6451  GAGGGTGGCG GTTCTGAGGG TGGCGGCTCT GAGGGAGGCG GTTCCGGTGG
      CTCCCACCGC CAAGACTCCC ACCGCCGAGA CTCCCTCCGC CAAGGCCACC

6501  TGGCTCTGGT TCCGGTGATT TTGATTATGA AAAGATGGCA AACGCTAATA
      ACCGAGACCA AGGCCACTAA AACTAATACT TTTCTACCGT TTGCGATTAT

6551  AGGGGGCTAT GACCGAAAAT GCCGATGAAA ACGCGCTACA GTCTGACGCT
      TCCCCCGATA CTGGCTTTTA CGGCTACTTT TGCGCGATGT CAGACTGCGA
```

ClaI
~~~~~~

6601 AAAGGCAAAC TTGATTCTGT CGCTACTGAT TACGGTGCTG CTATCGATGG
TTTCCGTTTG AACTAAGACA GCGATGACTA ATGCCACGAC GATAGCTACC

6651 TTTCATTGGT GACGTTTCCG GCCTTGCTAA TGGTAATGGT GCTACTGGTG
AAAGTAACCA CTGCAAAGGC CGGAACGATT ACCATTACCA CGATGACCAC

6701 ATTTTGCTGG CTCTAATTCC CAAATGGCTC AAGTCGGTGA CGGTGATAAT
TAAAACGACC GAGATTAAGG GTTTACCGAG TTCAGCCACT GCCACTATTA

6751 TCACCTTTAA TGAATAATTT CCGTCAATAT TTACCTTCCC TCCCTCAATC
AGTGGAAATT ACTTATTAAA GGCAGTTATA AATGGAAGGG AGGGAGTTAG

6801 GGTTGAATGT CGCCCTTTTG TCTTTGGCGC TGGTAAACCA TATGAATTTT
CCAACTTACA GCGGGAAAAC AGAAACCGCG ACCATTTGGT ATACTTAAAA

6851 CTATTGATTG TGACAAAATA AACTTATTCC GTGGTGTCTT TGCGTTTCTT
GATAACTAAC ACTGTTTTAT TTGAATAAGG CACCACAGAA ACGCAAAGAA

6901 TTATATGTTG CCACCTTTAT GTATGTATTT TCTACGTTTG CTAACATACT
AATATACAAC GGTGGAAATA CATACATAAA AGATGCAAAC GATTGTATGA

HindIII

6951 GCGTAATAAG GAGTCTTGAT A
CGCATTATTC CTCAGAACTA T
~~~~~~

Figure 5

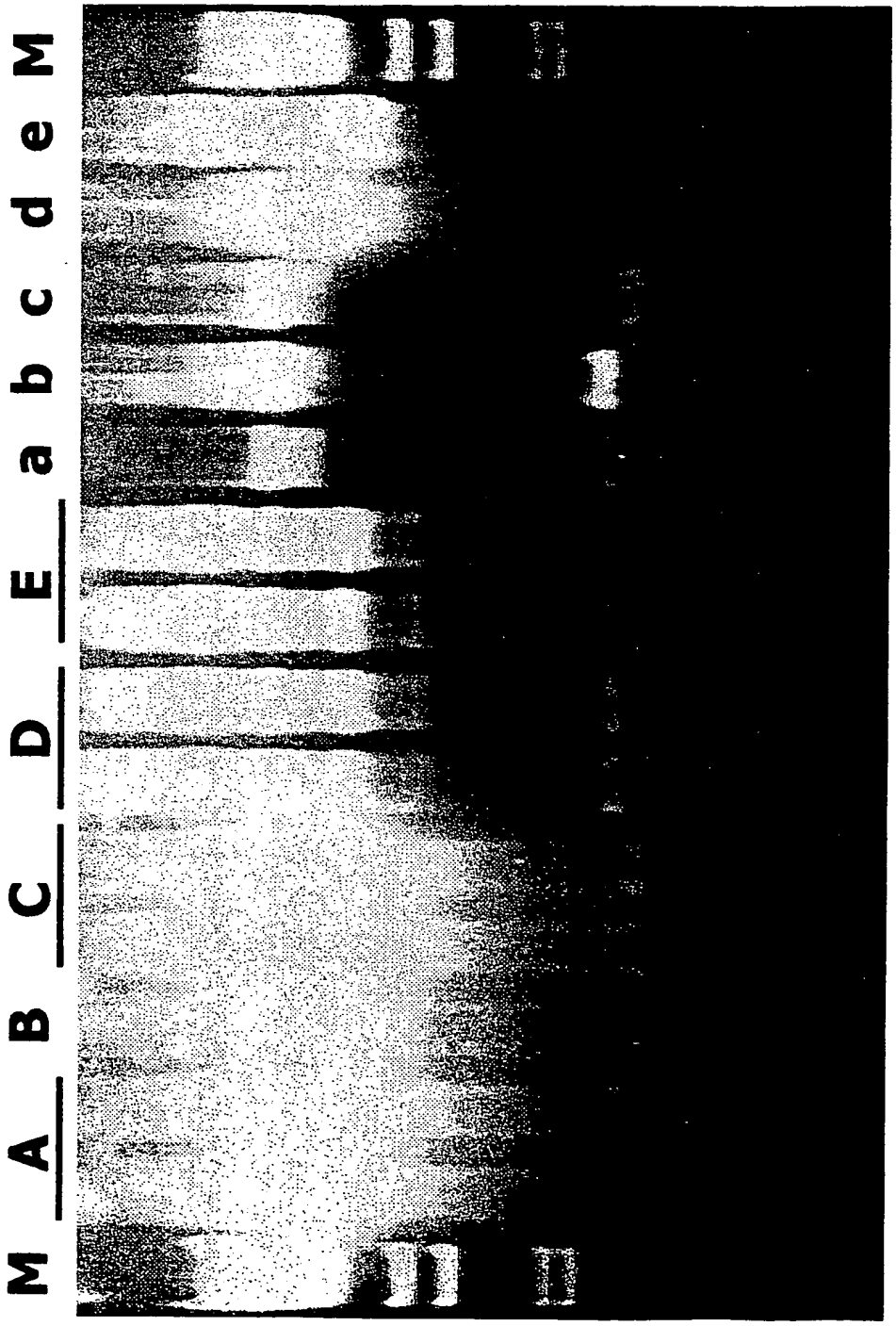


Figure 6

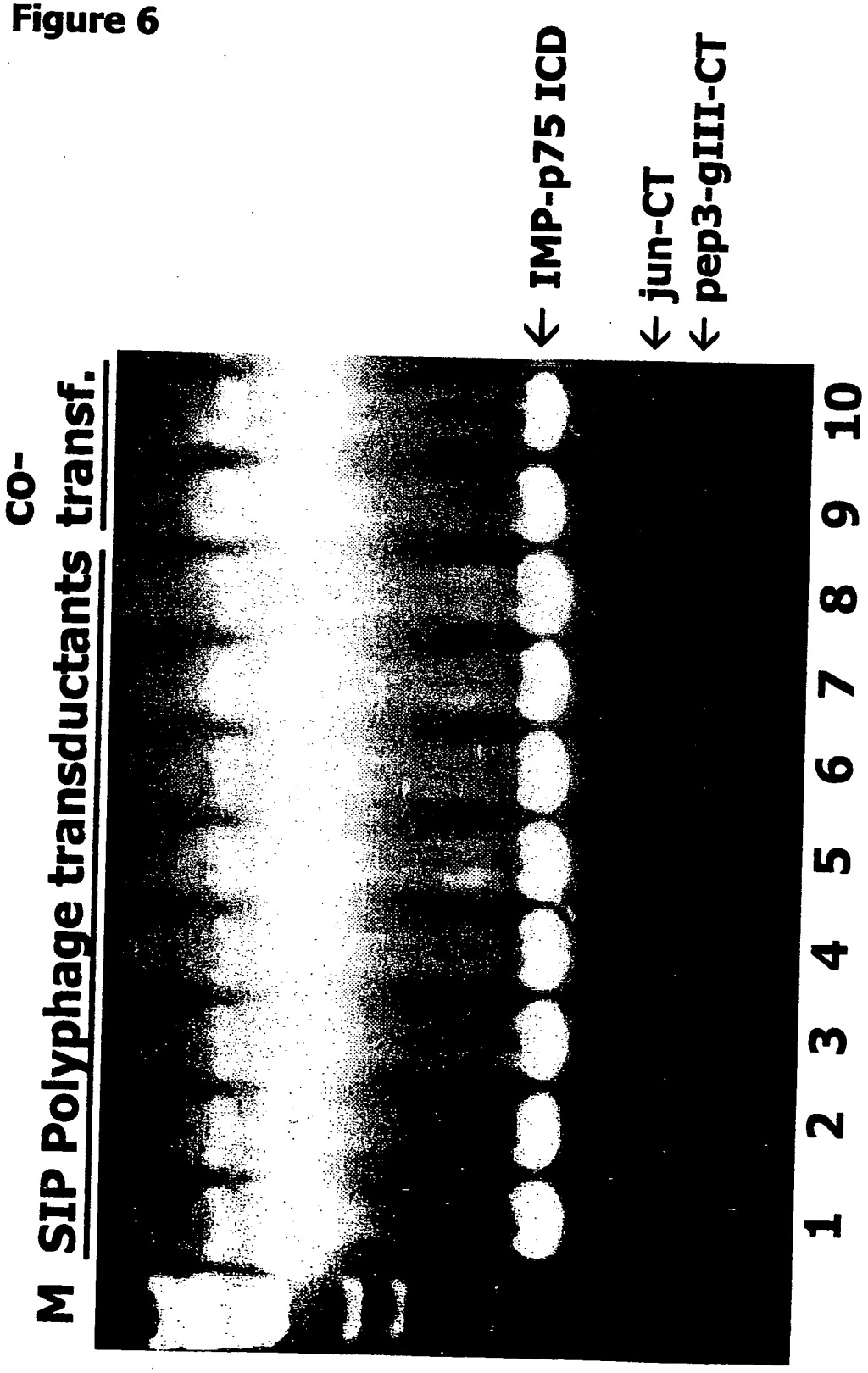

← IMP-p75 ICD
← jun-CT
← pep3-gIII-CT
M SIP Polyphage transductants co-transf.
1 2 3 4 5 6 7 8 9 10

Figure 7

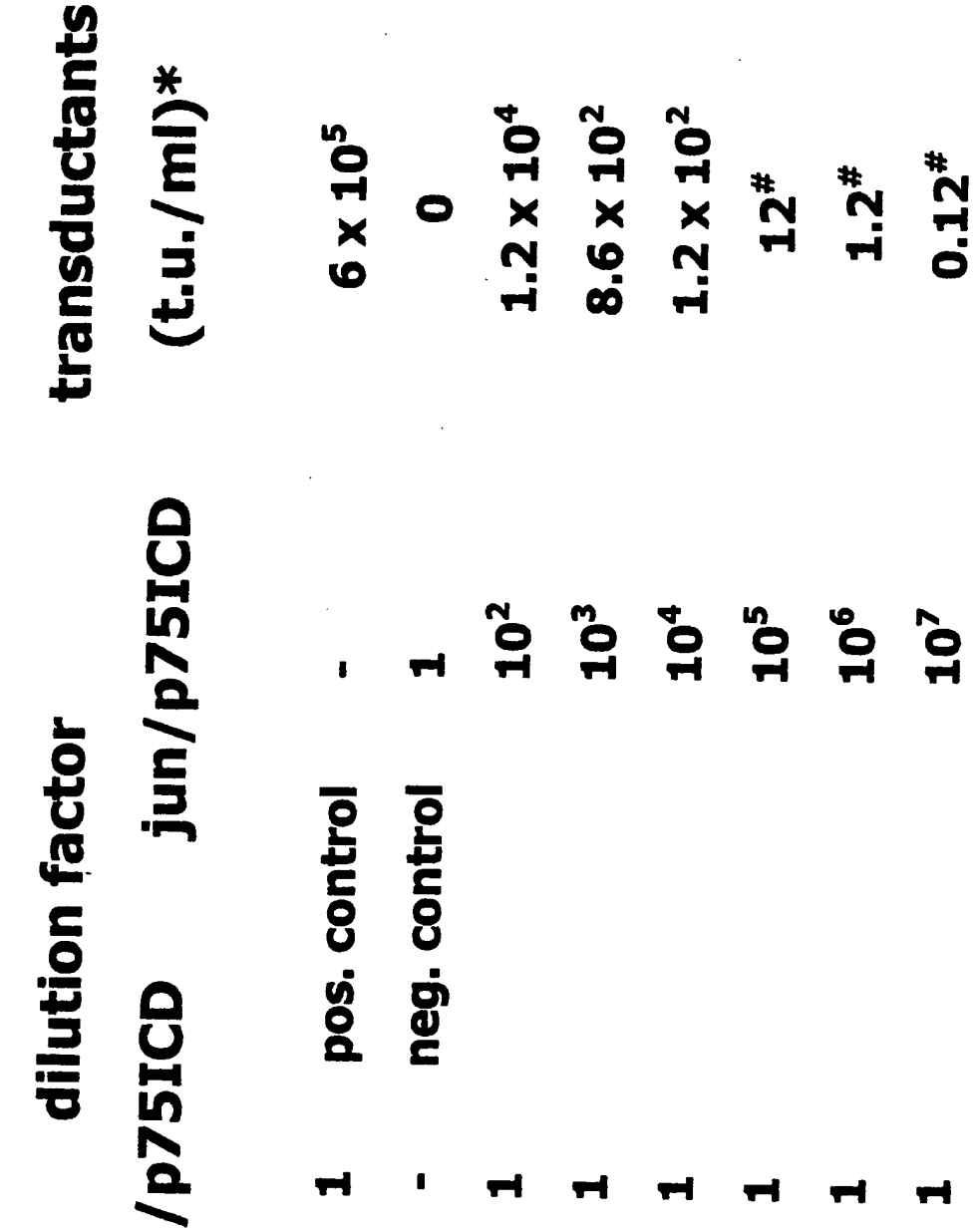

dilution factor
pep3/p75ICD
jun/p75ICD
transductants
(t.u./ml)*
1 pos. control - 6 x 10^5
- neg. control 1 0
1 10^2 1.2 x 10^4
1 10^3 8.6 x 10^2
1 10^4 1.2 x 10^2
1 10^5 12#
1 10^6 1.2#
1 10^7 0.12#

Figure 8

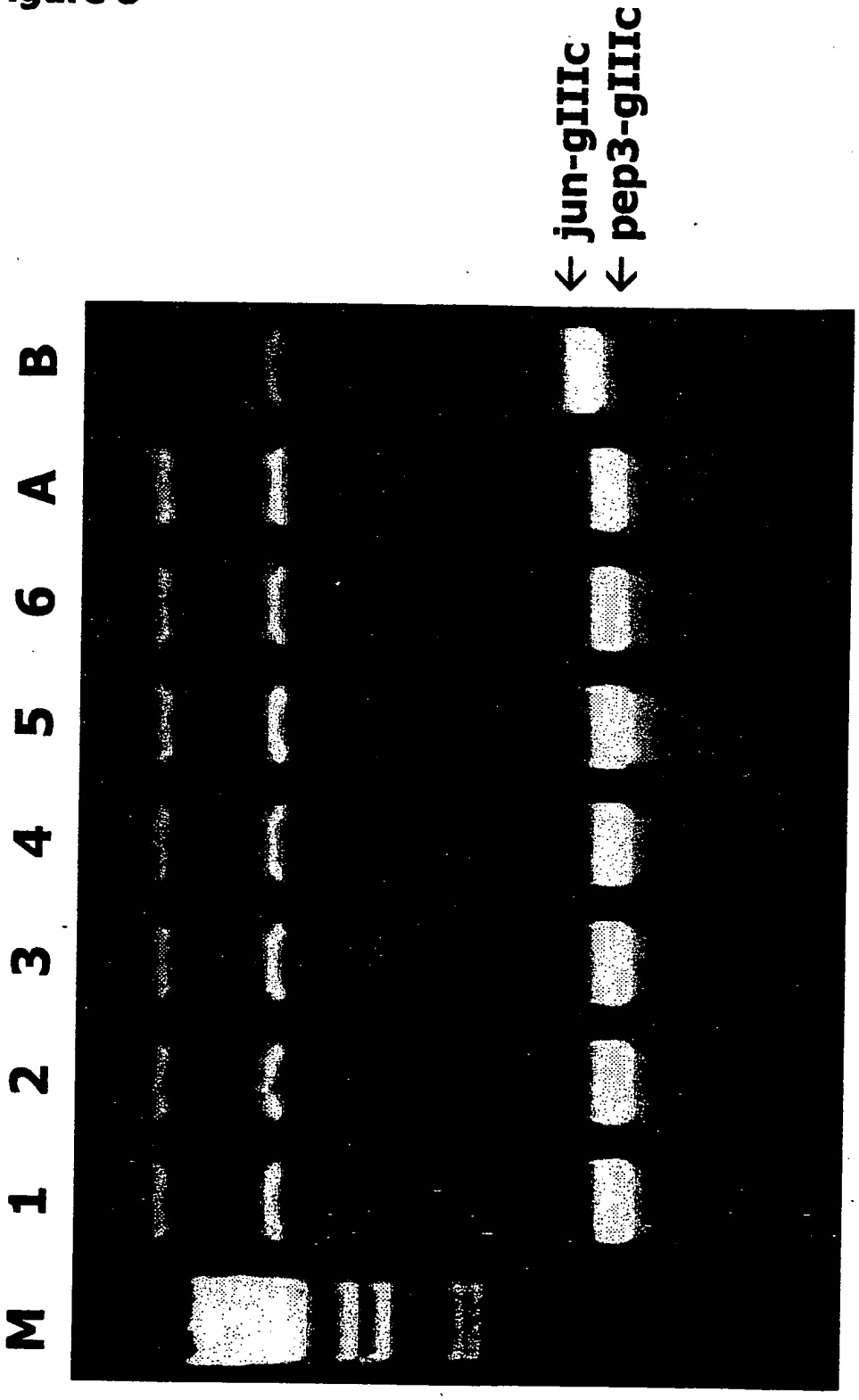


Figure 9

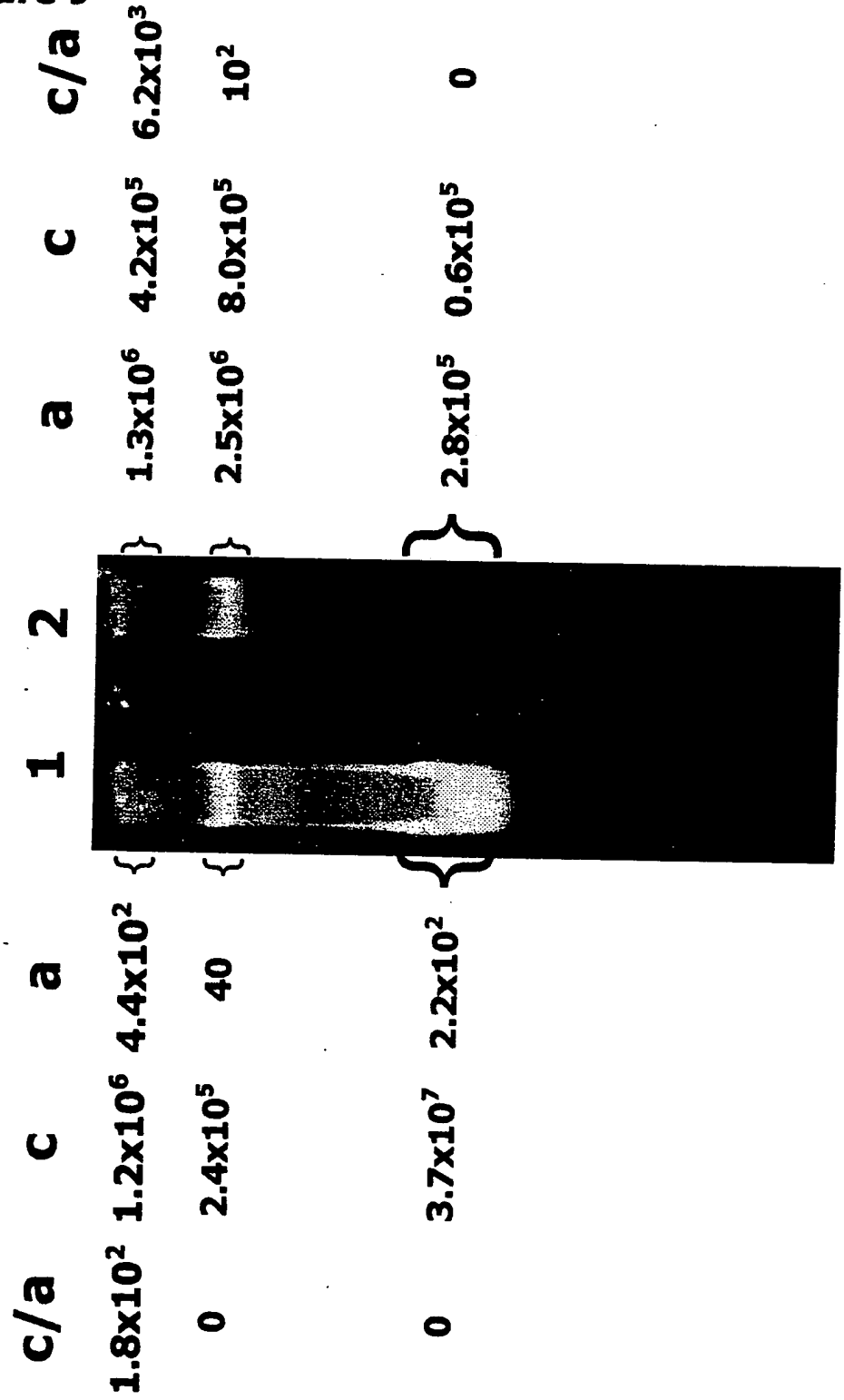

c/a
6.2x10³
10²
0
c
4.2x10⁵
8.0x10⁵
0.6x10⁵
a
1.3x10⁶
2.5x10⁶
2.8x10⁵
2
1
c/a
1.8x10²
0
0
c
1.2x10⁶
2.4x10⁵
3.7x10⁷
a
4.4x10²
40
2.2x10²

Figure 10